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[Management Brief]

An Evaluation of the Precision of Fin Ray, Otolith, and Scale Age Determinations for Brook Trout

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Abstract.—The ages of brook trout *Salvelinus fontinalis* are typically estimated using scales despite a lack of research documenting the effectiveness of this technique. The use of scales is often preferred because it is nonlethal and is believed to require less effort than alternative methods. To evaluate the relative effectiveness of different age estimation methodologies for brook trout, we measured the precision and processing times of scale, sagittal otolith, and pectoral fin ray age estimation techniques. Three independent readers, age bias plots, coefficients of variation (CV = 100 × SD/mean), and percent agreement (PA) were used to measure within-reader, among-structure bias and within-structure, among-reader precision. Bias was generally minimal; however, the age estimates derived from scales tended to be lower than those derived from otoliths within older (age > 2) cohorts. Otolith, fin ray, and scale age estimates were within 1 year of each other for 95% of the comparisons. The measures of precision for scales (CV = 6.59; PA = 82.30) and otoliths (CV = 7.45; PA = 81.48) suggest higher agreement between these structures than with fin rays (CV = 11.30; PA = 65.84). The mean per-sample processing times were lower for scale (13.88 min) and otolith techniques (12.23 min) than for fin ray techniques (22.68 min). The comparable processing times of scales and otoliths contradict popular belief and are probably a result of the high proportion of regenerated scales within samples and the ability to infer age from whole (as opposed to sectioned) otoliths. This research suggests that while scales produce age estimates rivaling those of otoliths for younger (age < 3) cohorts, they may be biased within older cohorts and therefore should be used with caution.

Scale-based age estimation techniques have been the prevailing methodology utilized by fisheries scientists in the estimation of freshwater fish age since the early part of the 20th century (Carlander 1987). Scales have gained favor as they are nonlethal, easy to collect, and believed to require less time and resources than age estimates produced by boney structures (Secor et al. 1992). However, evidence suggests that scale techniques may underestimate fish age within slow-growing populations and among older fish (Carlander 1987; Hining et al. 2000). Age estimation via boney structures such as otoliths, while lethal and potentially more time and resource intensive, may produce superior estimates, especially within older cohorts (Secor et al. 1992; Kruse et al. 1997). Most recently, fin ray techniques have been developed and utilized to estimate fish age (Beamish 1981; Hubert et al. 1987). Fin ray techniques are nonlethal and have the potential to produce superior age estimates in relation to the alternate nonlethal approach, scales. This is due, in part, to research suggesting that fin ray annuli are formed in isolation from fish physiology and are therefore potentially resistant to reabsorption (Simkiss 1974). Among salmonids, however, research comparing the relative effectiveness of age estimates produced by scale and fin ray techniques is generally inconsistent (Silkstrom 1983; Chilton and Bilton 1986; Hubert et al. 1987; Graynoth 1996).

The ages of brook trout *Salvelinus fontinalis* have historically been estimated using scales (Cooper 1951; Alvord 1953; Reimers 1958; Hatch 1961) and, more recently, otoliths (Hall 1991; Toetz et al. 1991). However, modern studies comparing these techniques in brook trout are rare. Magnan and Fitzgerald (1983) noted 92% agreement between scale and otolith age estimates, while Kozel and Hubert (1987) found only 27% agreement. Recently, however, the computation of percent agreement has waned with the application of more rigorous statistical and graphical approaches (Campana et al. 1995). Published reports of brook tout age estimation via fin ray techniques or the inclusion of this technique into a comparative study are lacking.

The objectives of this research were to apply modern graphical and statistical methods to evaluate the precision of scale, otolith, and fin ray age estimation techniques for brook trout. To further evaluate their utility, we will estimate the time required to collect, prepare, and infer age from the three structures.

Methods

Study site.—The research was conducted on Big Run, Back Run, Sawmill Branch, and Vance Run, which are located within the Monongahela National Forest, Pendleton County, West Virginia. These streams are all first- and second-order tributaries of

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the North Fork of the South Branch of the Potomac River and are inhabited predominantly by brook trout and mottled sculpin *Cottus bairdii*. The sites were chosen because they have naturally reproducing populations of brook trout and are not actively stocked with this species. The majority of land cover within the watersheds is mixed deciduous hardwood forest.

Fish sampling.—Brook trout were collected during the spring (March 11–13) of 2006. Young of the year had probably emerged by this time but were, however, too small to be effectively captured and therefore were not included within this study. Individuals were collected using a pulsed-DC backpack electrofishing unit and a one-pass removal technique. All individuals were immobilized in a 120-mg/L solution of clove oil and ethanol (Anderson et al. 1997), weighed to the nearest gram using a spring scale, and measured to the nearest millimeter total length. Once collected, inclusion into the study was based upon a specific sampling protocol. Such a protocol is warranted to minimize the number of individuals that have to be sacrificed and to enable sampling of individuals from the range of lengths exhibited within the population. Obtaining a representative sample assures that (1) all cohorts within the population are represented and (2) the variability in length within these cohorts is accounted for. The latter is especially important as growth at age probably influences scale morphology and thus the precision of age estimates (Bilton 1975). A representative sample which spans the range of lengths within each cohort will account for any within-cohort differences in structure precision that may exist as a result of size. To do this, as fish were collected, fish length was continuously fed into a length frequency histogram and an effort was made to collect five fish within each 10-mm size bin. Individuals who fell within a size bin that required additional sampling were sacrificed in a 500-mg/L solution of clove oil and ethanol, frozen, and transported back to West Virginia University laboratories for hard part removal. Individuals falling within size bins that did not require additional sampling were placed in a live well and allowed to recover fully before being released close to the point of capture. To minimize impact, no more than 40 fish were sacrificed from any one stream.

Hard part preparation.—In the laboratory, fish were allowed to thaw, and scales were removed posterior to the dorsal fin and above the lateral line (DeVries and Frie 1996). Scales (20–30) from each fish were wet mounted on a glass slide and viewed with transmitted light under a microscope at 45×. Both sagittal otoliths were removed following the "open the hatch" method (Secor et al. 1992), cleaned, dried, and stored in plastic vials. If necessary, otoliths were sanded gently (800 grit), then polished (1,200 grit) with sand paper to clarify the annuli. Both sagittae were viewed whole under a dissection scope at 12× using reflected light. Right pectoral fins were removed, dried, and mounted in Epofix embedding resin. Mounted fin rays were cross-sectioned (0.5 mm) with an Isomet variable-speed precision saw equipped with a 127-mm-diameter diamond wafering blade rotating at 300 revolutions per minute (Beamish and Chilton 1977). Several consecutive fin ray sections were mounted to a glass slide using crystal bond and viewed with transmitted light under a microscope at 45×. Total removal and processing time was recorded for all hard parts.

Age estimation.—Age estimates were produced by three independent readers trained in annulus identification. Each reader estimated fish age from scales, otoliths, and fin rays without knowledge of fish length following the method of DeVries and Frie (1996). Fin ray age estimates were derived from the first or second ray of the most easily read cross section. To maintain independence, samples within the data set were randomized before each reading, and no reader made multiple readings of the data set within the same day. Total data set reading time for each structure was recorded at the end of each age estimation session.

Statistical analysis.—Age bias plots (Campana et al. 1995), percent agreement (PA), and coefficients of variation (CV = 100 × SD/mean; Chang 1982; Campana et al. 1995) were used to investigate within-reader, among-structure bias and among-reader, within-structure precision. Bias must first be addressed before precision can be measured owing to possible systematic over- or underestimation of ages. If one structure consistently produces different ages than another, estimates of precision will be biased and may lead to misinterpretation. To detect bias, age bias plots were created for each within-reader, among-structure comparison. Compared with previous visual methods of evaluating bias, such as scatter and age difference plots, age bias plots are more sensitive to nonlinear bias across age-classes (Campana et al. 1995). Age bias plots depict the mean age assigned by one reader for all fish assigned a given age by a second reader. For example, if reader A determines that five fish are all age 1 but reader B assigns them ages 1, 2, 2, 3, and 3, the mean age assigned by reader B would be 2.2. The selection of reader for the abscissa is arbitrary. Each age-class contains 95% confidence intervals which enable interpretation of deviance from the 1:1 equivalence line. Either parallel or separated lines of increasing divergence indicate bias between the two age estimations. As errors may be nonlinear, visual examination is the most effective method to explore potential bias (Campana et al. 1995). For unbiased results, precision was estimated for each structure using CV as

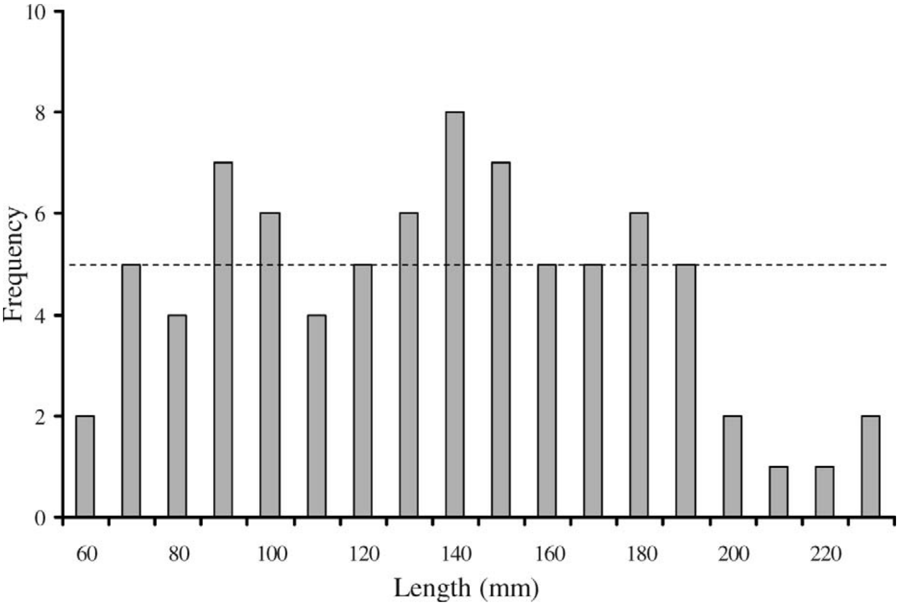

FIGURE 1.—Length frequency histogram for the brook trout included within the study of age estimation methods. The dashed line indicates the desired number of individuals within each size bin according to the sampling protocol.

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

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 age is estimated. The CV is averaged across samples for each structure to produce a mean CV.

To investigate the amount of effort required to generate ages from each structure, the total removal, preparation, and reading time in minutes was summed. The per-sample processing time for each structure was computed as the quotient of the total number of samples within the data set and total processing time. Multiple readings of each structure facilitated testing for differences in mean reading time. Differences were investigated using analysis of variance (ANOVA) with a post hoc Tukey test completed in SAS version 9.1 statistical software package (SAS Institute 2003) at $\alpha = 0.05$.

Results

A total of 83 fish were collected and sacrificed from the four sample streams. Two samples were removed prior to analysis—one owing to a unique hard part shape that may have resulted in non-independent readings and the other to the inability to find a nonregenerated, readable scale within the sample. Altogether, 81 individuals ranging from 61 to 239 mm in length and encompassing ages 1 through 4 were included in the analysis (Figure 1). However, because of the scarcity of very large (>200-mm) and small (<70-mm) individuals, the collection criterion (five fish per 10-mm size bin) was not met for size bins below 70 mm and above 190 mm.

The age bias plots of within-reader, among-structure comparisons showed minimal bias (Figure 2). That is, the equivalence and empirical lines did not exhibit extensive parallelism or increasing divergence. Fin rays tended to give lower ages than otoliths and scales for the oldest (age-4) cohort and higher ages for the youngest cohort (age 1). Scales also tended to underestimate ages assigned by otoliths but for fish greater than 2 years of age. While the age bias plots identified inconsistencies, none persisted in more than two consecutive cohorts or represented a difference greater than 1 year and could effectively be ignored for precision estimates. Otolith–scale comparisons (PA = 81.48; CV = 6.77) exhibited the best mean estimates of precision, while scale–fin ray (PA = 69.96; CV = 10.81) and otolith–fin ray (PA = 67.90; CV = 10.98) comparisons were less precise. Among-reader, within-structure measures of precision indicate that scale-based age estimations were only slightly more precise than otolith estimations as evidenced by mean PA and mean CV estimates (Table 1). Fin rays exhibited the poorest precision (Table 1). However for all comparisons, ages were within 1 year of each other for 95% of the fish.

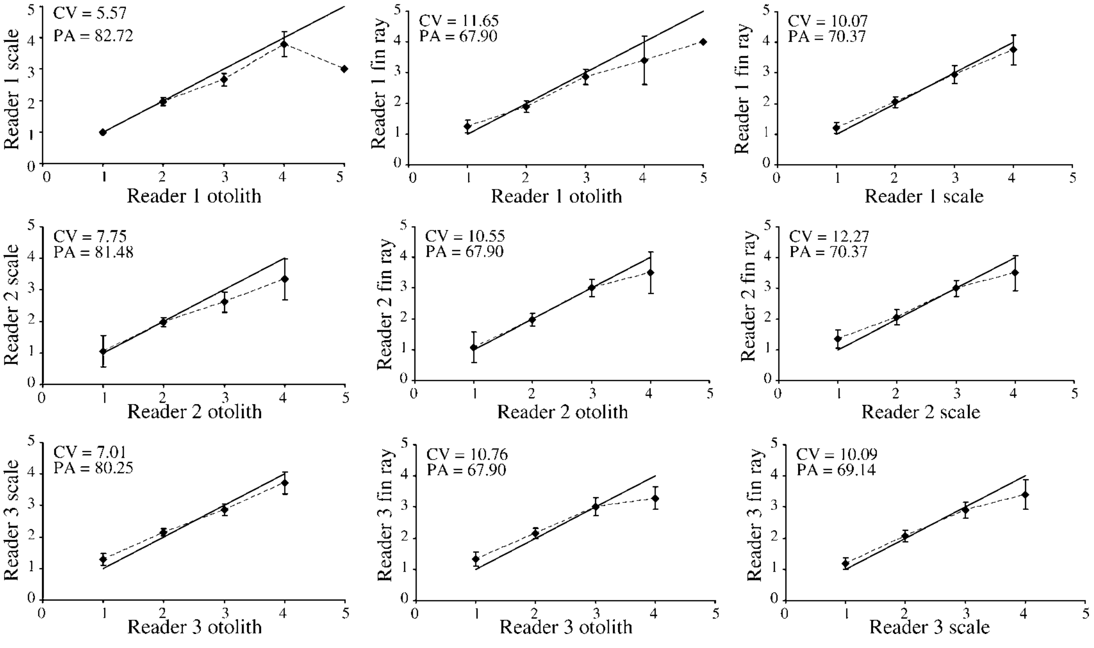

FIGURE 2.—Age bias plots with 95% confidence intervals (for points with multiple observations) showing within-reader, among-structure comparisons. Bias is indicated by increasing divergence between the empirical (broken) lines and the equivalence (solid) lines.

Hard part removal and preparation time varied substantially with structure (Table 2). In comparison to otoliths, scale techniques benefited from decreased removal time; however, preparation and reading times were both greater. Fin ray techniques exhibited the greatest per-sample processing time as a result of the mounting and sectioning process. Once prepared, however, fin ray sections required the least amount of time for inferring age. The ANOVA indicated no significant differences between structure reading times ($F = 4.86$; $P = 0.057$). However, a post hoc Tukey test indicated that scale reading time was significantly greater than fin ray reading time ($P < 0.05$).

Discussion

Investigations into bias reveal that scales tend to produce age estimates lower than those of otoliths among older individuals, as has been reported for brook trout and other salmonid species (Silkstrom 1983; Hining et al. 2000). In this research, scale underestimation was apparent within cohorts 3 and 4, and was probably a result of seasonal or ontogenetic alterations in growth, thus restricting, eroding, or potentially removing annular marks within these cohorts (Carlander 1974; Simkiss 1974). Within younger (age < 3) cohorts, however, scale and otolith age estimations were more consistent. Similar results were found by Hining et al. (2000) and Silkstrom (1983) who noted that scale age estimation techniques of rainbow trout *Oncorhynchus mykiss* and Arctic grayling *Thymallus arcticus* broke down after the age of 2. The bias in the fin ray–scale and fin ray–otolith

TABLE 1.—Precision of scales, otoliths, and fin rays in the determination of brook trout ages, as indicated by the mean percent agreement (PA) and mean coefficient of variation (CV) of among-reader, within-structure comparisons.

Metric	Scales	Otoliths	Fin rays
Mean PA	82.30	81.48	65.84
Mean CV	6.59	7.45	11.30

TABLE 2.—Total removal and preparation times, mean reading time, and per-sample processing time (min) for structures used in the estimation of brook trout age. Processing time per sample was derived by dividing the sum of the removal, processing, and mean reading times by the number of samples ($N = 83$) within the data set.

Structure	Removal	Preparation	Mean reading[a]	Processing time per sample
Scale	35	967	122 z	13.88
Otolith	532	359	100 zy	12.23
Fin ray	54	1709	74 y	22.68

[a] Different letters signify significant differences ($P < 0.05$).

comparisons was less consistent. Previous fin ray research suggests that failure to identify the first annuli may be common, potentially leading to underestimation (Beamish 1973). This is one possible explanation for lower fin ray age relative to otolith age within the age-4 cohort. However, evidence of greater disagreement (both over- and underestimations) across cohorts for fin ray–scale, fin ray–otolith, and within-structure, among-reader fin ray comparisons suggests that chronic misidentification of the first annuli may only be part of the problem. Fin ray annuli were often ill defined, most notably within younger (age $<$ 3) individuals. While annuli clarified with age, the presence of, and necessity to differentiate and tally greater numbers of, annuli within older cohorts probably led to errors. Combined, these characteristics often made fin ray age estimation difficult and, in all likelihood, inconsistent.

Investigations of precision reveal scale techniques to be slightly more precise than otolith techniques and both to be more precise than fin ray techniques. In regards to scale and otolith precision, the finding of increased precision of scale techniques relative to otoliths is contrary to some salmonid age estimation studies (Silkstrom 1983; Graynoth 1996; Kruse et al. 1997). However, this result is probably an artifact of the limited number of larger and older fish included in the research. The inclusion of greater numbers of these individuals will probably reduce overall scale precision estimates as previous research, as well as results presented here, suggest that scales tend to produce poorer estimates of fish age within older cohorts (Silkstrom 1983; Hining et al. 2000).

Contrary to the common belief, scale techniques did not entail less processing time than otolith techniques. This was most likely because of the small scale size and prominence of regenerated scales. Upwards of 80% regenerated scales were common within samples, especially among older (age $>$ 2) cohorts. To those not familiar with brook trout scales, this does not pose a problem for field collection as generally 50 scales can be removed by the single swipe of a blade. However, once in the laboratory it becomes increasingly time-consuming to find readable scales within that sample. Alternatively, otolith processing time was dramatically reduced, as annuli were clearly discernable without the need to mount and section each sample. Aside from reading time, fin ray techniques required the greatest amount of time to process. Evidence of significantly shorter fin ray reading times in relation to scale techniques is probably a result of the sectioning process. However, the overall greater processing time of fin rays, combined with their lower precision, argue against their use with brook trout.

In conclusion, the research presented here suggests that while scales produce age estimates rivaling those of otoliths for younger (age $<$ 3) cohorts, they may underestimate otolith age within older (age $>$ 2) age-classes. As older and larger individuals probably have increased ecological relevance, garnering accurate data on these individuals becomes important and may preclude the use of scales in favor of otoliths. However, as brook trout populations may be dominated by younger age-classes, evidence of the utility of scale-based estimation techniques within these cohorts should not be ignored (Cooper and Scherer 1967; Whitworth and Strange 1983). In reference to fin ray techniques, their utility was not demonstrated within this research. However, as they have proved valuable in similar salmonid studies (Shirvell 1980; Silkstrom 1983), we hesitate to denounce their use in brook trout but rather use this opportunity to call for additional research to refine techniques. As always, decisions regarding age estimation techniques should be made only after research goals and data quality needs are accessed, so that the influence of potential deficiencies is minimized.

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