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MANAGEMENT BRIEFS

Precision and Bias of Largemouth, Smallmouth, and Spotted Bass Ages Estimated from Scales, Whole Otoliths, and Sectioned Otoliths

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Abstract.—We compared the precision and bias of age estimates derived from scales, whole otoliths, and sectioned otoliths and by readers of varying experience level for largemouth bass *Micropterus salmoides,* smallmouth bass *M. dolomieu,* and spotted bass *M. punctulatus* from Skiatook Lake, Oklahoma. Precision was assessed with the coefficient of variation of age estimates for each fish. Bias was determined among readers and structures from age bias graphs derived from least squares regression. Precision was similar among species for all three structures and among structures for any species, except for smallmouth bass; its age estimates were more precise with whole otoliths. Bias between pairs of readers was found for scales and whole otoliths among all three species but never for sectioned otoliths. The experience level of the reader influenced the bias between readers for scales but not for otoliths. Bias between structures was found between scales and whole otoliths for all three species and between scales and sectioned otoliths for smallmouth bass and spotted bass. Age estimates were unbiased between whole and sectioned otoliths for all three species. Although sectioned otoliths required more preparation time, they provided the best age estimates for the three populations in Skiatook Lake.

Age estimation is essential to fish population management. The age structure of a population can be used to quantify growth and total mortality, as well as assess environmental or management impacts (DeVries and Frie 1996). It is therefore imperative that fisheries managers and researchers use the best available methods to estimate age of their target species.

Scales have traditionally been used to age black bass (DeVries and Frie 1996), but estimates using these structures have often proven to be inaccurate and difficult to repeat (Erickson 1983; Boxrucker 1986; Welch et al. 1993). As an alternative to scales, otoliths increasingly are being used; however, they require sacrificing fish and often take more preparation time (Hoyer et al. 1985; Howells 1994; DeVries and Frie 1996). In older fish, otoliths may require sanding or sectioning to distinguish annuli and to estimate age with confidence (Hoyer et al. 1985).

Annual ring deposition in otoliths has been verified for largemouth bass *Micropterus salmoides* (Taubert and Tranquilli 1982) and smallmouth bass *M. dolomieu* (Heidinger and Clodfelter 1987) but not for spotted bass *M. punctulatus.* Annual ring formation in scales of spotted bass, however, has been verified through increment analysis (Olmsted and Kilambi 1978). We assumed that annual rings form in otoliths of spotted bass, as they do in otoliths of largemouth bass and smallmouth bass.

Our objective was to quantify and compare the precision of age estimates for largemouth bass, smallmouth bass, and spotted bass as derived from scales, whole otoliths, and sectioned otoliths. Using Skiatook Lake, Oklahoma, to provide the study specimens, we assessed bias for these species between all possible pairs of readers (reader bias) and structures (structure bias). Additionally, we wanted to verify that growth of the black bass

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populations in Skiatook Lake is typical of other southeastern reservoirs.

Methods

Study site.—Skiatook Lake, a 4,266-ha impoundment of Hominy Creek in Osage County, Oklahoma, was formed in 1984 by the U.S. Army Corps of Engineers primarily for flood control. The lake has a mean depth of 9.7 m, a shoreline development ratio of 11.3, and a Secchi disk visibility in summer of 173 cm in the main pool (Oklahoma Department of Wildlife Conservation, unpublished data). The lake perimeter consists largely of steep, bedrock substrate with little aquatic vegetation. Skiatook Lake was classified as mesotrophic in 1997, having a mean chlorophyll-*a* concentration of 5.49 mg/m³ (Long 2000).

Data collection.—We captured 138 largemouth bass, 136 smallmouth bass, and 195 spotted bass from Skiatook Lake by boat electrofishing in spring of 1997 and 1999. Scales from below the lateral line posterior to the operculum and sagittal otoliths were removed from each fish, and both structures were stored dry.

Scales were pressed onto acetate slides, and otoliths were mounted in EMBed 812 (Electron Microscopy Sciences Fort Washington, Pennsylvania). Scale impressions were examined on a microfiche reader at 32× magnification. Otoliths were examined whole in the embedding medium with a dissecting scope at 20–40× magnification with reflected light, and after sectioning (250 μm thick) in the transverse plane with a low-speed diamond blade saw, the sections were similarly examined in immersion oil. All structures were examined once by three independent readers without prior knowledge of the ages estimated by the other readers, the length of the fish, or the species of fish. Because management agencies sometimes rely on ages estimated by inexperienced readers, such as students, we included estimates of age from readers with two levels of experience. Reader 1 (J. M. Long) was experienced at estimating ages of fish using all three structures, whereas readers 2 and 3 had no previous experience with any of the structures but were trained by reader 1 and allowed to practice with each structure before estimating ages for the study.

Statistical methods.—Precision was assessed with the coefficient of variation (CV) of age estimates (Chang 1982). The CV of each *j*th fish is the ratio of the standard deviation to the mean and was computed as

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

where X_{ij} is the *i*th age of the *j*th fish, X_j is the mean age of the *j*th fish, and R is the number of times each fish is aged. The CV can be averaged among many fish to produce a mean and standard deviation. Because the CV estimate requires the mean age of a particular fish in the denominator, we added one to all ages to avoid dividing by zero. Low values for CV indicate high levels of precision. We used analysis of variance (ANOVA) to test whether CV estimates were significantly different among species within structures and among structures within species. For significant differences, we used LSD to determine where those differences occurred.

Reader bias was assessed using the graphical methods of Campana et al. (1995) by constructing age bias graphs. Age bias graphs plot the average ages estimated by one reader for all fish against the age determined by a second reader (Campana et al. 1995). Bias was determined for all possible pairs of readers by regressing the mean age estimated by the inexperienced reader (readers 2 and 3) onto the age estimated by the experienced reader (reader 1) and by regressing the mean age estimated by one inexperienced reader (reader 3) onto the age estimated by the other inexperienced reader (reader 2).

To assess bias between structures, we computed the modal age of each structure for each fish. We then constructed age bias graphs between all possible pairs of structures for each species using the method for reader bias. However, for this method we plotted the average modal age for one structure against the modal age estimated by another structure for all fish. We then used *t*-tests for each age bias graph to test the null hypotheses that (1) the intercept (β_0) was equal to zero, and (2) the slope (β_1) was equal to one (indicating 1:1 agreement between readers or structures). Rejection of either hypothesis was interpreted as bias in the age estimates. Only those ages represented by three or more fish were included in the regression analyses.

To verify that the black bass populations in Skiatook Lake are similar to other black bass populations throughout the southeastern United States, we obtained back-calculated length-at-age data, based on scales, from reservoirs in Kentucky, Tennessee, and Virginia and compared those graphs

TABLE 1.—Mean coefficient of variation (CV) of largemouth bass, smallmouth bass, and spotted bass ages estimated by three independent readers from scales, whole otoliths, and sectioned otoliths.

Structure	*N*	Mean CV	SD of CV
	Largemouth Bass		
Scales	130	13.2369	14.6238
Whole otoliths	130	11.478	13.4946
Sectioned otoliths	130	15.199	15.5563
	Smallmouth Bass		
Scales	134	13.0597	14.6785
Whole otoliths	134	8.9875	12.8011
Sectioned otoliths	134	13.3101	14.0702
	Spotted Bass		
Scales	185	12.4002	13.0561
Whole otoliths	185	11.6933	13.8789
Sectioned otoliths	185	14.1279	14.4927

with similar data from Skiatook Lake. Back-calculated lengths-at-age for the Skiatook Lake populations were determined using the Fraser-Lee formula in DisBCal software (Frie 1982). Because DisBCal does not include an intercept for spotted bass, we substituted the intercept for smallmouth bass.

Results

Precision

We found no significant differences in CV among species for ages estimated from scales ($F_{2,\,446}$ = 0.16, P = 0.853), whole otoliths ($F_{2,\,446}$ = 1.79, P = 0.169), or sectioned otoliths ($F_{2,\,446}$ = 0.55, P = 0.578; Table 1). However, when comparing among structures for each species, smallmouth bass age estimates were more precise using whole otoliths than those using scales or sectioned otoliths ($F_{2,\,399}$ = 4.10, P = 0.017; Table 1). For largemouth bass and spotted bass age estimates, all structures were equally precise ($F_{2,\,387}$ = 2.12, P = 0.122 and $F_{2,\,552}$ = 1.52, P = 0.220, respectively; Table 1).

Reader Bias

Bias between readers was evident for all three species, but fewer cases were found with smallmouth bass (Figures 1–3). Largemouth bass and spotted bass age estimates from scales were biased in two of three comparisons but in only one comparison for smallmouth bass. Whole otoliths were biased in two of three comparisons for largemouth bass and in one comparison for smallmouth bass and spotted bass. We found no indication of reader bias for any species using sectioned otoliths.

The level of experience played a role in assessing bias, which was most notable with scales. When using scales, bias between experienced and inexperienced readers was evident in five of six cases for all three black bass species but not between both inexperienced readers (Figure 1–3). With whole otoliths, bias was evident in two of six cases between experienced and inexperienced readers and in two of three cases between the two inexperienced readers. There was no bias due to experience level when sectioned otoliths were used.

Structure Bias

Bias in age estimation between whole otoliths and scales was found for all species (Figure 4). Compared to whole otoliths, scales underestimated ages of older fish and overestimated ages of younger fish. Structure bias was also evident between scales and sectioned otoliths for smallmouth bass and spotted bass and was similar to the bias for scales and whole otoliths (Figure 4). However, structure bias was not evident between scales and sectioned otoliths for largemouth bass. No age bias was present for any species when sectioned otoliths were compared to whole otoliths (Figure 4).

Growth

Mean back-calculated lengths at age of largemouth bass, smallmouth bass, and spotted bass in Skiatook Lake was moderate in comparison to the populations in the other five southeastern reservoirs (Figure 5). Additionally, spotted bass exhibited the slowest growth of the species in all reservoirs. Except for the Pickwick Reservoir population, largemouth bass grew the fastest of the three species. In Pickwick Reservoir, smallmouth bass exhibited the fastest growth.

Discussion

Except for smallmouth bass age estimates from whole otoliths, precision among species and structures was statistically similar, which indicates that readers were identifying and counting the same annuli regardless of species or structures. We believe the increased precision of age estimates using whole otoliths for smallmouth bass was related to otolith structure. The dorsal edges of the otoliths from the smallmouth bass in our study were not smooth like those from largemouth bass and spotted bass but consisted of tooth-like projections (teeth). Because the annuli for these species of black bass form in late spring (Olmstead and Kilanbi 1978; Taubert and Tranquilli 1982; Heidinger and Clodfelter 1987) and our sampling was con-

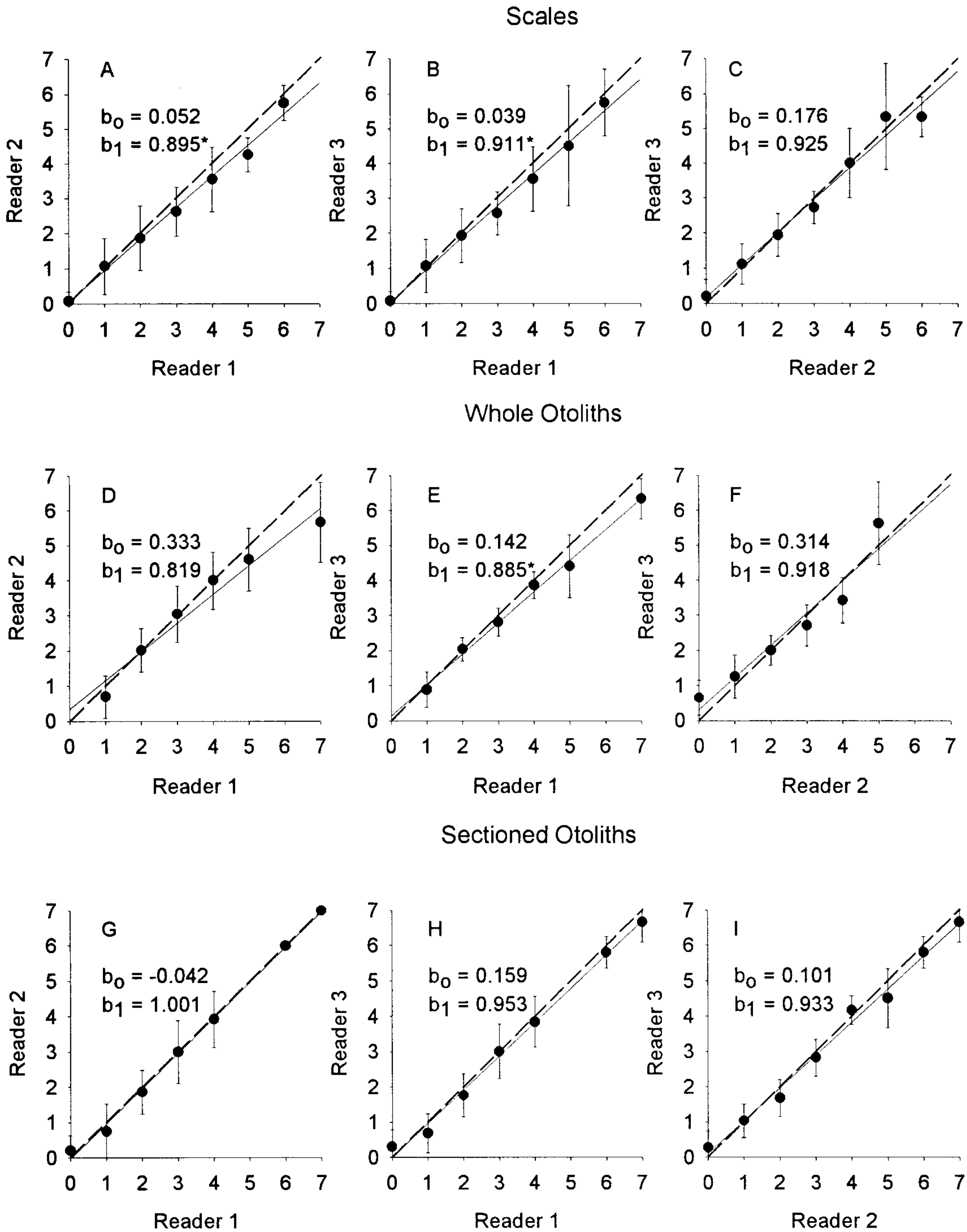

FIGURE 1.—Age bias graphs for largemouth bass age estimates from scales, whole otoliths, and sectioned otoliths between all possible pairs of comparisons between three readers. Dashed line indicates 1:1 agreement in age estimates between the two readers (*X* axis and *Y* axis) being compared. Solid line is the least squares regression line. Error bars are 1 SD around the mean age assigned by reader *Y* for every fish assigned an age by reader *X*. An asterisk indicates a significant difference ($P \leq 0.05$) from 0 (zero) for the estimate of the *y*-intercept (b_0) and a significant difference from 1 for the estimate of the slope (b_1). Fish were collected from Skiatook Lake, Oklahoma, in 1997 and 1999.

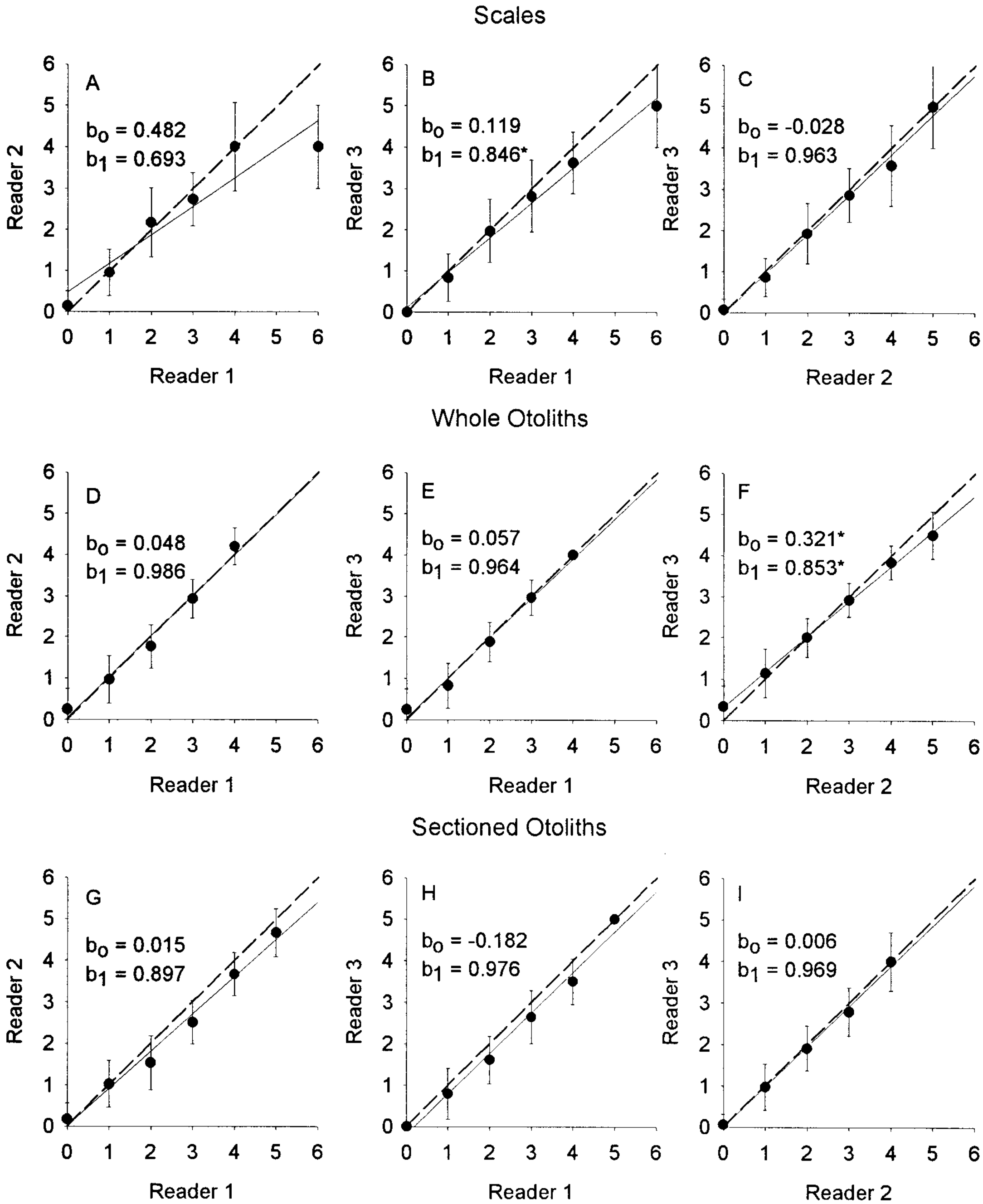

FIGURE 2.—Age bias graphs for smallmouth bass age estimates from scales, whole otoliths, and sectioned otoliths between all possible pairs of comparisons between three readers. Dashed line indicates 1:1 agreement in age estimates between the two readers (*X* axis and *Y* axis) being compared. Solid line is the least squares regression line. Error bars are 1 SD around the mean age assigned by reader *Y* for every fish assigned an age by reader *X*. An asterisk indicates a significant difference ($P \leq 0.05$) from 0 (zero) for the estimate of the *y*-intercept (b_0) and a significant difference from 1 for the estimate of the slope (b_1). Fish were collected from Skiatook Lake, Oklahoma, in 1997 and 1999.

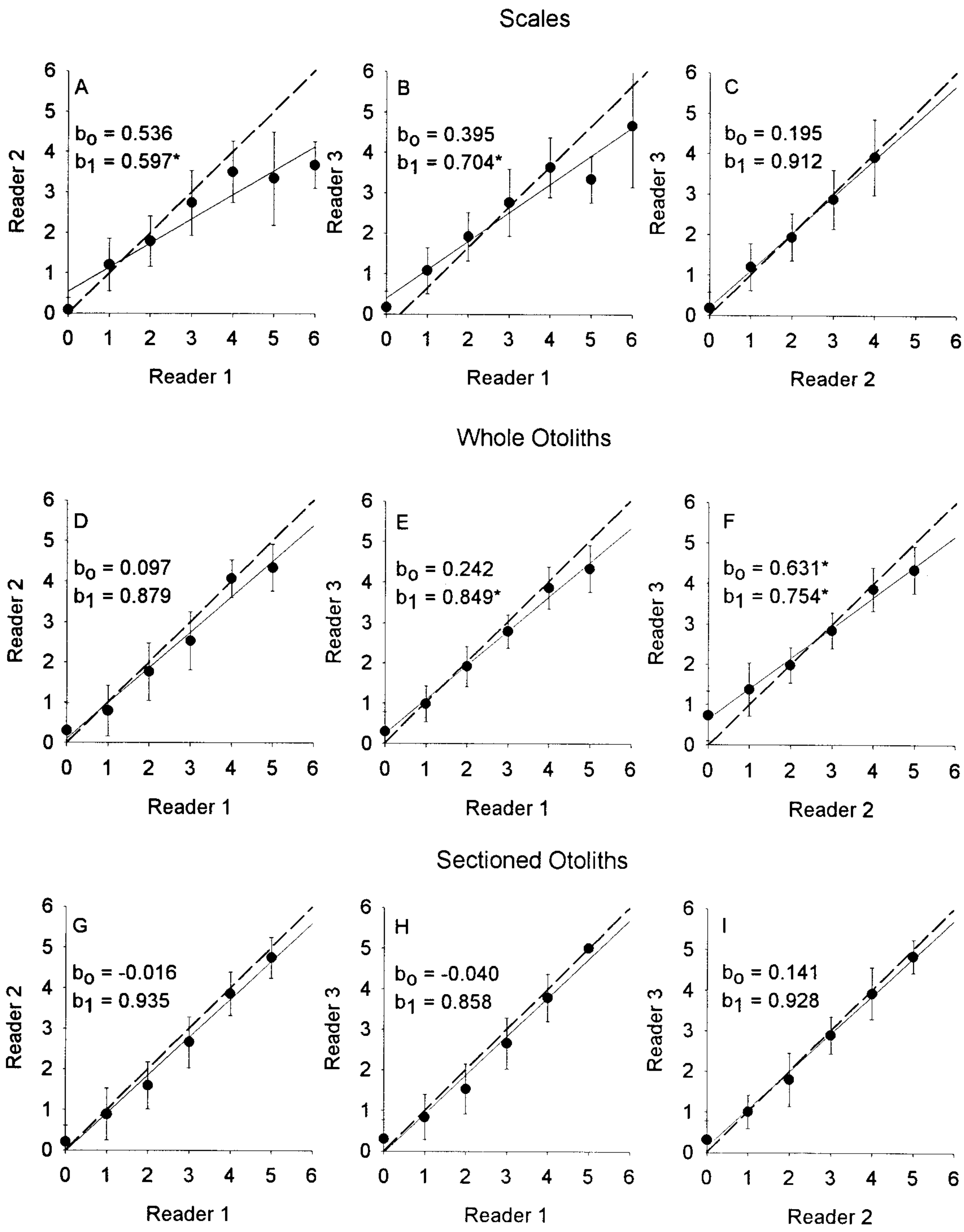

FIGURE 3.—Age bias graphs for spotted bass age estimates from scales, whole otoliths, and sectioned otoliths between all possible pairs of comparisons between three readers. Dashed line indicates 1:1 agreement in age estimates between the two readers (*X* axis and *Y* axis) being compared. Solid line is the least squares regression line. Error bars are 1 SD around the mean age assigned by reader *Y* for every fish assigned an age by reader *X*. An asterisk indicates a significant difference ($P \leq 0.05$) from 0 (zero) for the estimate of the *y*-intercept (b_0) and a significant difference from 1 for the estimate of the slope (b_1). Fish were collected from Skiatook Lake, Oklahoma, in 1997 and 1999.

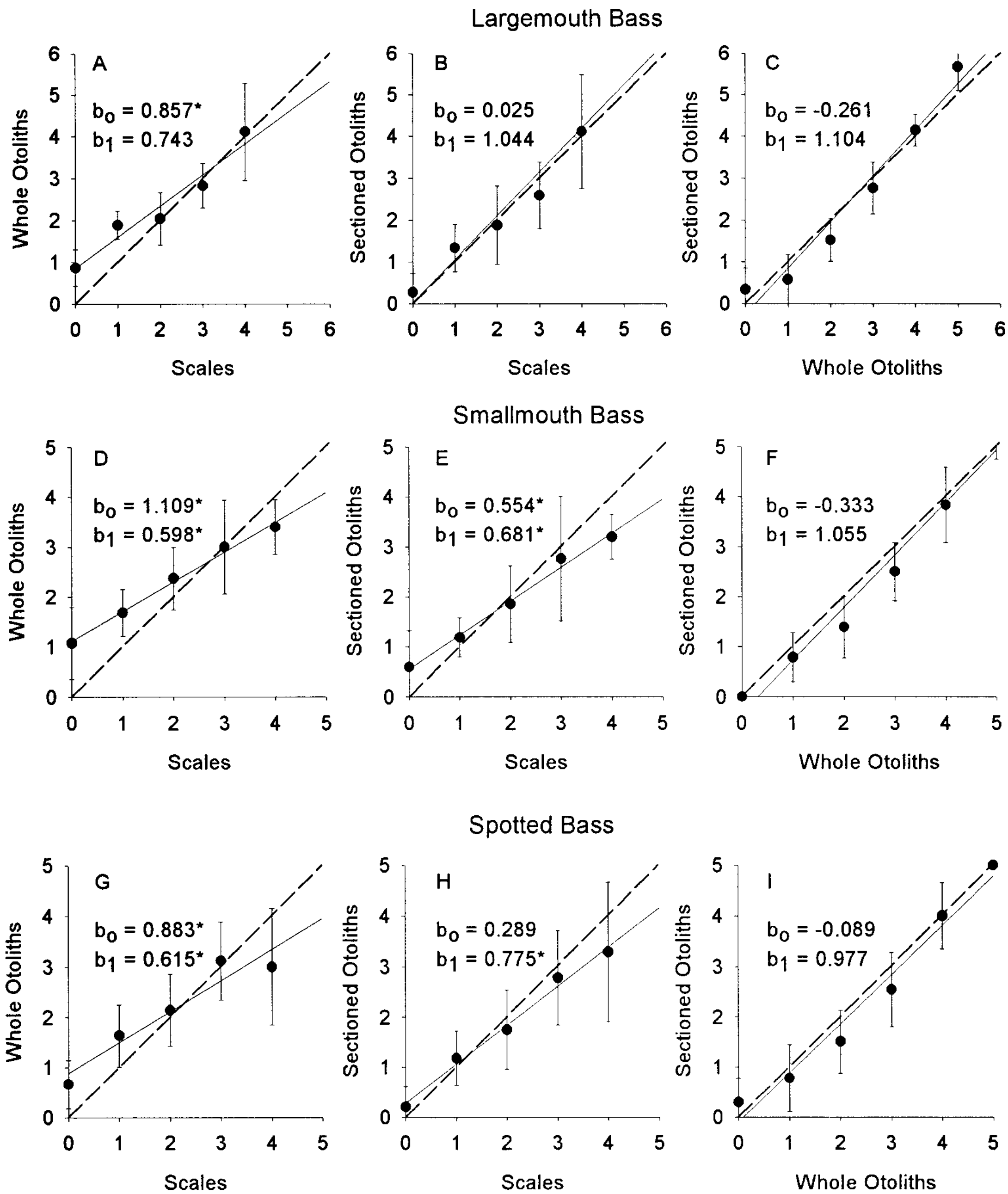

FIGURE 4.—Age bias graphs for age estimates from largemouth bass, smallmouth bass, and spotted bass for comparisons between all possible pairs of three structures used for aging. Dashed line indicates 1:1 agreement in age estimates between structures *Y* and *X* (axes). Solid line is the least squares regression line. Error bars are 1 SD around the mean modal age assigned by structure *Y* for every fish assigned a modal age by structure. An asterisk indicates a significant difference ($P \leq 0.05$) from 0 (zero) for the estimate of the *y*-intercept (b_0) and a significant difference from 1 for the estimate of the slope (b_1). Fish were collected from Skiatook Lake, Oklahoma, in 1997 and 1999.

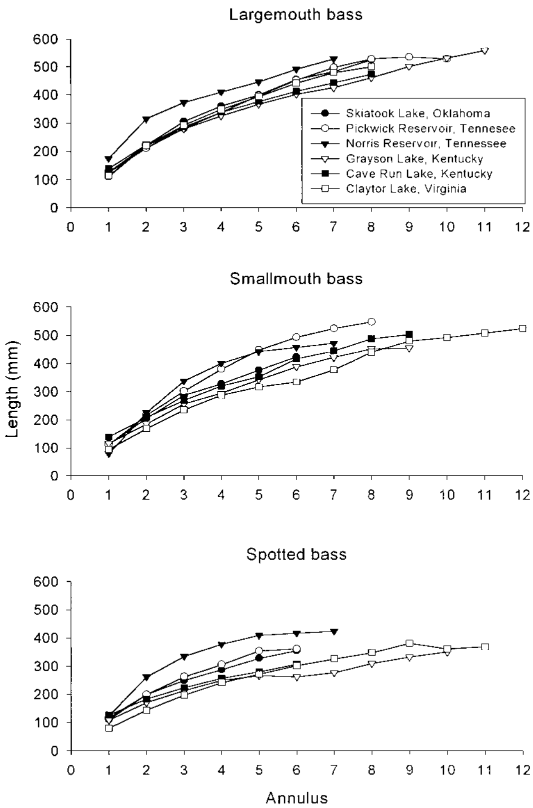

FIGURE 5.—Mean back-calculated length at annulus (estimated from scales) of largemouth bass (LMB), smallmouth bass (SMB), and spotted bass (SPB) in Skiatook Lake, Oklahoma; Pickwick Reservoir, Tennessee (Hubert 1976); Norris Reservoir, Tennessee (Stroud 1948); Grayson Lake, Kentucky (Gerard Buynak, unpublished data); Cave Run Lake, Kentucky (Gerard Buynak, unpublished data); and Claytor Lake, Virginia (Craig Bonds, unpublished data).

ducted from April to June, the last annulus was entirely apparent in later samples but not in earlier samples. However, in the earlier samples a portion of the last annulus could be discerned in the anterior edge of the otoliths of largemouth bass and spotted bass and in the teeth of the dorsal edge of the otoliths of smallmouth bass. Because the dorsal edge of the otolith is larger than the anterior edge, it is reasonable that the last annulus was more likely to be counted in whole otoliths of smallmouth bass taken from all samples, early and late, and thus increased the precision of age estimates for this species in our study.

Bias between pairs of readers indicated that not all annuli were being identified similarly when scales and whole otoliths were used, regardless of species, and these biases probably influenced the precision of age estimates (Campana et al. 1995). Additionally, bias due to the experience level of the reader was evident when scales were used. Because there was no reader bias with sectioned otoliths, we conclude that sectioned otoliths are the most reliable structure to estimate age for these three black bass species in Skiatook Lake. Moreover, the lack of reader bias with sectioned otoliths indicates the usefulness of this method, regardless of the experience level of the reader.

These results are especially applicable to inexperienced readers (i.e., college students) and fisheries biologists who do not regularly estimate fish ages. Campana and Moksness (1991) found that accuracy of daily age estimates from otoliths of larval herring *Clupea harengus* was positively associated with experience level of the reader and that inexperienced readers who polished their otoliths obtained more accurate age estimates than inexperienced readers using unpolished otoliths. Conversely, they found reader experience level did not significantly affect precision of age estimates, although a trend was apparent (Campana and Moksness 1991). Our results suggest that level of experience needs to be considered when analyzing data from these different structures or that selection of the structure used for estimating ages of these black bass species should be based on the experience level of the reader(s). For example, much more training would be needed for inexperienced readers estimating ages from scales and less training would be needed for sectioned otoliths. If experienced readers were available and fish could not be sacrificed, then our results suggest that scales would provide adequate age estimates, at least for largemouth bass up to age 4.

Hoyer et al. (1985) recommended the use of sectioned otoliths over whole otoliths, especially for older and slower growing fish, because they provided the most accurate estimates. However, Howells (1994) found that sectioned otoliths underestimated age of largemouth bass 6–8 years of age in Texas. Heidinger and Clodfelter (1987) showed that scales were only accurate for 71% of age estimates of smallmouth bass in Illinois but that broken otoliths, which were similar to our otolith sections, were 100% accurate. There is a paucity of literature on age estimation of spotted bass, and otoliths have not been validated for this species.

We believe that our results for the black bass populations in Skiatook Lake are applicable to these species in other southeastern reservoirs of the United States. Growth can affect the ability to estimate age (Hoyer et al. 1985). Because the mean back-calculated lengths at annulus for all black bass species in Skiatook Lake were moderate, compared to the other populations that we examined, we conclude that our results are applicable to these other systems.

The preferred structure for estimating ages of fish varies with species, locality, size, and growth rate, and recommending one structure over another may not work for all situations. Boxrucker (1986) recommended using whole otoliths for estimating ages of white crappie *Pomoxis annularis* in Oklahoma but noted that scales may be just as precise for populations of this species in Missouri. Beamish (1979) preferred sectioned otoliths to whole otoliths for older fish because they provided more accurate estimates. Boucher (1998) recommended using sectioned otoliths over scales to detect the presence of older largemouth bass and smallmouth bass in Maine but concluded that scales were reliable up to age 5 and could be used for routine management purposes. For largemouth bass in Skiatook Lake, ages up to age 4 were similarly estimated from scales and sectioned otoliths. Additionally, scale estimates were similar to sectioned otolith estimates up to age 3 for spotted bass and from age 1 to age 3 for smallmouth bass; thus, scales may be an alternative to using otoliths for these age classes. However, our results at Skiatook Lake show scales were less precise than sectioned otoliths because they were affected by reader bias and level of experience. Therefore, we recommend that sectioned otoliths be used, when possible, to estimate the age of largemouth bass, smallmouth bass, and spotted bass in southeastern United States reservoirs because otoliths provide the most accurate and least biased age estimates. Additionally, we recommend further research to validate the otolith method for estimating ages of spotted bass.

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