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Evaluation of age estimates made with scales, dorsal spines, and whole otoliths as surrogates for ages estimated with halved otoliths of Smallmouth Bass and Largemouth Bass in Minnesota

by

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Abstract — We evaluated scales, dorsal spines, and whole sagittal otoliths from Smallmouth Bass and Largemouth Bass as surrogate aging structures for halved otoliths. Because we lacked known-age bass, we assumed that ages estimated with annuli counts on halved otoliths were the most accurate because this aging method was validated as accurate in other studies. Two sets of independent age estimates were made with each age structure collected from five populations of Smallmouth Bass and 12 populations of Largemouth Bass from Minnesota waters. Whole otolith ages of Smallmouth Bass estimated by both readers usually matched (> 80% agreement) halved otolith ages 1 through 3 whereas whole otolith ages of Largemouth Bass usually equaled ages 2 to 4 estimated with halved otoliths. Whole otoliths underestimated age of bass older than age 4 because they became too thick to reveal innermost annuli. Scale ages estimated by the more experienced reader usually equaled halved otolith ages 1 through 3, but estimates made by the less experienced reader were unreliable for all ages. The less experienced reader often counted as annuli one or more false annuli commonly found on scales of both species; thus, estimates were often positively biased with respect to halved otolith ages. Conversely, dorsal spine ages estimated by both readers were negatively biased with respect to halved otolith ages except age 1. Dorsal spines of both species possessed central lumens that obscured the earliest annuli, and typical crowding of annuli on the spine edge also occurred. Between-reader and between-structure agreement decreased with increasing age of bass, and they often differed among populations. Overall, we recommend using halved or sectioned otoliths to estimate age of both bass species in Minnesota; however, whole otoliths and scales provide useful estimates of age for younger (< age 4) bass if estimates are made by competent agers.

Smallmouth Bass *Micropterus dolomieu* and Largemouth Bass *M. salmoides* are highly sought sportfish in Minnesota, and effective management of these bass fisheries requires accurate estimates of age. Most age estimates of both bass species made by staff from the Minnesota Department of Natural Resources (MNDNR) are based on annuli counts on scales, but some estimates are made by counting annuli on sagittal otoliths (both whole view and cross-sectioned) and dorsal spines (McInerny et al. in press). However, with the exception of cross-sectioned or halved otoliths, few studies exist that evaluated the utility of these structures for estimating age of these bass species in Minnesota and elsewhere.

Many studies suggest that ages estimated with sectioned or halved otoliths provide the most accurate and precise age estimates of Smallmouth Bass and Largemouth Bass. Marks on halved or sectioned otoliths have been validated as annuli for Smallmouth Bass up to age 4 and for Largemouth Bass up to age 16 (Taubert and Tranquilli 1982; Hoyer et al. 1985; Heidinger and Clodfelter 1987; Buckmeier and Howells 2003; Taylor and Weyl 2013). However, sectioned otolith age estimates agreed less than 50% of the time with known-age 6 through 8 Largemouth Bass from Texas ponds, but annual draining of ponds and handling of test fish could have affected annulus formation on otoliths (Howells et al. 1997).

Presumably, MNDNR staff would prefer reading whole otoliths rather than halved or sectioned otoliths because of faster processing; however, other studies report variable reliability of ages estimated with whole views. Estimating age via whole views of otoliths of Walleye *Sander vitreus* required half the time needed for estimating age via halved otoliths (Isermann et al. 2003). Long and Fisher (2001) found similar among-reader precision in age estimates of an Oklahoma population of Largemouth Bass made with whole otoliths and those with sectioned otoliths but found better among-reader aging precision with whole otoliths than sectioned otoliths of Smallmouth Bass from the same reservoir. They also did not observe aging bias between these two structures for ages 1 through 5 Smallmouth Bass and ages 1 through 7 Largemouth Bass. Over 90% of ages of Largemouth Bass in North Carolina waters estimated with whole otoliths equaled those estimated with sectioned otoliths, but sectioned otolith ages exceeded whole otolith ages of Largemouth Bass ≥ 347 mm TL (Besler 2001). Conversely, Maceina and Sammons (2006) found that annuli on whole otoliths from Smallmouth Bass and Largemouth Bass from the Hudson River, New York, became less distinguishable when ages exceeded 6 or 7. Based on halved otoliths, both species of bass in Minnesota can exceed age 7 (MNDNR lake survey database); thus, whole otolith ages could become unreliable in older bass.

In many cases, MNDNR staff cannot or will not sacrifice bass to obtain otoliths; thus, they need to know the range of ages that can be estimated with scales or dorsal spines because these latter two structures can be obtained without sacrificing bass. Scales show promise as aging structures for some northern populations of Smallmouth and Largemouth bass, but evaluations of this structure are rare. Scale ages up to age 7 were validated as accurate for Largemouth Bass from an Ontario lake, and annulus formation was validated on ages 3, 4, and 5 Smallmouth Bass in a Wisconsin lake (Maraldo and MacCrimmon 1979; Klumb et al. 1999). In warmer latitudes, estimates of both bass species made with scales for populations in Illinois, Oklahoma, North Carolina, Texas, Alabama, and South Africa were either inaccurate or biased compared to known-ages or sectioned-otolith ages older than age 2 (Prather 1966; Prentice and Whiteside 1974; Heidinger and Clodfelter 1987; Howells et al. 1997; Besler 2001; Long and Fisher 2001; Taylor and Weyl 2012).

Evaluations of dorsal spines as aging structures for Smallmouth Bass and Largemouth Bass are rare and inconclusive; thus, additional evaluations are needed. Maraldo and MacCrimmon (1979) reported that age estimates based on annuli counts on dorsal spine cross sections often did not agree with estimates made with scales or whole otoliths from an Ontario population of Largemouth Bass. However, Sotola et al. (2014) reported better agreement and precision among readers of dorsal spines than they did for scales from New York populations of Smallmouth Bass and Largemouth Bass. None of these aging structures from either bass species from Minnesota waters has been evaluated for precision or accuracy. Over a thousand bass fisheries occur throughout Minnesota; thus, we have the opportunity to test if precision and bias of age estimates differ among populations. Estimation of accuracy is unattainable because known-age bass populations do not exist in Minnesota; thus, our primary objective was to determine if ages estimated with scales, dorsal spines, and whole otoliths are similar to those made with halved otoliths because the latter has been validated elsewhere as being accurate. Even though most studies show that estimates of age based on scales and dorsal spines are less precise or biased with respect to estimates from halved or sectioned otoliths, this study will provide a range of ages that can be reliably estimated with these structures. Our last objective was to determine if precision of scale age, dorsal spine age, and whole otolith age and their agreement with halved otolith ages differ among populations of bass in Minnesota because there could be opportunities to use non-lethal or less costly methods for some bass populations.

METHODS

Collection and processing of aging structures

Attempts were made to collect both Smallmouth Bass and Largemouth Bass from different regions of Minnesota. Bass were captured with either boom electrofishing, gill netting, or trap netting. Total lengths (TL) in mm of each bass were measured, and scale samples were removed from the left side in a region just posterior of the depressed pectoral fin. In addition, the two to three of the most anterior dorsal spines were cut at the skin surface and removed from each bass, and both sagittal otoliths were also removed. All structures were usually placed in labeled coin envelopes and dried; however, otoliths of Largemouth Bass from Lake Carlos were placed in vials rather than coin envelopes.

Impressions of scales (three scales per bass) were made on clear acetate, and impressions were magnified with the aid of a microfiche reader. Whole otoliths were immersed in water in a clear dish, placed on a black stage plate, illuminated with a fiber-optic light, and magnified with the aid of a stereo microscope. Several weeks to a few months after estimates with whole views were completed, otoliths were then snapped in half along the transverse plane by applying pressure with a thumbnail, and broken edges were then placed over a flame until they turned golden brown (Barber and McFarlane 1987). These halved otoliths were then placed in clay (burnt side up), coated with a drop of mineral oil, placed under a stereomicroscope, and illuminated with a fiber optic light (Barber and McFarlane 1987). A low-speed circular saw was used to crosscut the proximal end of dorsal spines, and the distal end was impaled in clay or cardboard so that the spine sat upright. Each spine was then placed under a stereo microscope, and the spine shaft was illuminated with a fiber optic light. Cuts by the saw usually left sufficiently smooth surfaces on spine ends which negated sanding; however, adding mineral oil smoothed the proximal surface for viewing. Processing of scales, dorsal spines, and otoliths reflected that done by MNDNR staff (Logsdon 2007; McInerny et al. In press).

A total of four readers provided age estimates in this study. One reader provided a set of age estimates from each structure for each species, one reader provided one set of scale age estimates from each species, one reader provided one set of whole otolith (WO) age estimates of Largemouth Bass, and one reader provided a set of dorsal spine (DS) age estimates for each species, a set of halved otolith (HO) age estimates for each species, and a set of WO age estimates for Smallmouth Bass. Each reader worked at a different location; thus, microfiche readers and microscopes differed. For each structure, Reader 1 is the person who read the appropriate structure first, and Reader 2 supplied the second set of age estimates. Reader 2 usually examined the same otolith half and dorsal spine as Reader 1; however, Reader 2 could have read a different scale or whole otolith than Reader 1. Each reader estimated age at the time of capture, thus, sampling dates were available. Otherwise, readers lacked any other information (i.e., length-frequency distributions) that could help them estimate age.

Data analyses

We used two measurements of precision (percent agreement and mean coefficients of variation (CV) of age), age-bias analyses, and logistic linear mixed-effects modeling to evaluate each structure. We separated these analyses into two categories: between-reader analyses of scale, DS, WO, and HO age, and between-structure comparisons between scale age and HO age, DS age and HO age, and WO age and HO age.

We calculated for all samples combined, by age, and by population (each population is recognized as the water body where sampled) percent agreement of age estimates between readers of each structure for each species (Campana et al. 1995). When reporting percent agreement, we used 80% as a benchmark because this value represents the minimum acceptable agreement for many age structure evaluations (Maceina et al. 2007). We then calculated for each structure CV of the two age estimates of each bass made by both readers, and then calculated for each structure mean between-reader CV for all samples combined and for each population (Chang 1982). We then estimated age-bias between readers by estimating mean age ± 95% confidence limits of one reader as a function of the other for each aging structure from each species (Campana et al. 1995). Lastly, we used logistic linear mixed-effects models to explain the effects on between-reader agreement (either yes or no) of each individual bass potentially caused by population, mean age of each individual, and the interaction between population and mean age (agreement (structure x) = f (population, mean age (structure x), or population + mean age (structure x)). Mean age is the average age estimated by the two readers of the structure being evaluated. The variable ‘population’ was set as a random effect, and mean age was treated as a fixed effect. We used Akaike Information Criteria (AIC) to select the best fitting model explaining between-reader agreement for each aging structure and species. Modeling was accomplished with the *lme4* package in *R* (version 3.2.2) (Bates et al. 2015; R Core Team 2015).

We then tested to determine if scale age estimates, DS age estimates, and WO age estimates were the same as HO age estimates. To reduce the size of tables and figures for reporting, we calculated for each bass agreement and mean CV based on three age estimates (one scale, DS, or WO age estimated by one reader, and the two HO ages by Readers 1 and 2). We then calculated for each reader of scales, DS, and WO of each species, the percent of individuals that all three estimates were equal (percent agreement) and mean CV for all samples and for each population. We segregated these calculations by each HO reader if substantial age-bias occurred between HO readers.

We then estimated for each species age-bias as a function of HO age by calculating mean scale age, mean DS age, and mean WO age ± 95% confidence limits by each reader per HO age estimated by each HO reader (Campana et al. 1995). To reduce the number of plots by half, we averaged for each HO age the two means and two sets of confidence intervals for each structure unless clear age-bias occurred between HO readers.

Lastly, we used logistic linear mixed-effects models to explain relationships between population, mean HO age, and the population + mean HO age interaction on agreement between scale age by each reader and both HO ages, DS age by each reader and both HO ages, and WO age by each reader and both HO ages (agreement = f (population, mean HO age, or population + mean HO age). Mean HO age of each bass was calculated from the two HO estimates. The lowest AIC scores coupled with the fewest independent variables depicted the best model.

RESULTS

We collected age structures from 190 Smallmouth Bass ranging from 102 to 536 mm TL, and sample sizes ranged from 17 to 81 among the five water bodies (Table 1). Age structures were collected from 405 Largemouth Bass (total lengths ranged from 108 to 471 mm) from 12 lakes throughout Minnesota. Sample sizes of Largemouth Bass ranged from 10 to 68 per lake (Table 1).

TABLE 1. List of water bodies, their Minnesota Department of Natural Resources Division of Waters (DOW) number and sample size (n) of Smallmouth Bass and Largemouth Bass collected for age structure comparisons.

Water body	DOW number	n
Smallmouth Bass		
Two Island	16-0156-00	39
Bear	38-0405-00	23
Ten Mile	11-0413-00	17
Mississippi River – Little Falls		30
Mississippi River – Sauk Rapids		81
Largemouth Bass		
Echo	69-0615-00	24
Bear Head	69-0254-00	64
South Twin	44-0140-00	15
Portage	29-0250-00	21
Ten Mile	11-0413-00	22
Horseshoe	11-0358-00	49
Carlos	21-0057-00	68
Cedar	49-0140-00	44
Pearl	73-0037-00	31
Artichoke	06-0002-00	10
Peltier	02-0004-00	14
Madison	07-0044-00	43

Smallmouth Bass

Between-reader analyses of age structures

Between-reader analyses of age structures from Smallmouth Bass suggested that HO age estimates were the most precise and unbiased compared to estimates made with the other three structures. Percent age agreement was lowest and mean CV of age was highest between scale readers and the converse occurred between HO readers (Table 2).

Between-reader precision for all age structures of Smallmouth Bass was poor (% agreement < 80%; mean CV > 10%), declined with increasing age, and differed among populations. Agreement between scale readers was below 80% among all scale ages, declined with increasing scale age, and ranged from 6 to 47% among populations (Figures 1 and 2). Mean CV of scale ages ranged from 10 to 48% among populations (Figure 2). Between DS readers, agreement exceeded 80% at DS age 1 but also declined with increasing DS age (Figure 1). Between-reader agreement of DS age ranged from 23 to 72%, and mean CV of DS age ranged from 9 to 20% among the five populations (Figure 2). Agreement between WO readers usually exceeded 80% at WO ages 1 through 3, but agreement declined with increasing WO age when WO age exceeded age 3 (Figure 1). Agreement between WO ages ranged from 26 to 93% among populations, but exceeded 80% in only two populations (Figure 2). Mean CV of WO ages ranged from 0.6 to 18% among populations but was below 10% in four populations (Figure 2). Between-reader agreement of HO ages also usually exceeded 80% for HO ages 1 through 3, and agreement at higher HO age was usually lower than 80% (Figure 1). Between-reader agreement of HO ages ranged from 18 to 97% among populations but exceeded 80% in only two populations (Figure 2). Mean CV of HO ages ranged from 0.2 to 13% among populations and was lower than 4% in four populations (Figure 2).

TABLE 2. Percent agreement and mean coefficient of variation (CV) of age between two readers of scales, dorsal spines, whole otoliths, and halved otoliths, and mean percent agreement and mean CV between scale age, dorsal spine age, and whole otolith age by each reader (subscript $_1$ denotes Reader 1; subscript $_2$ denotes Reader 2) versus ages estimated with halved otoliths (both readers combined) of Smallmouth Bass and Largemouth Bass from Minnesota.

	Smallmouth Bass		**Largemouth Bass**	
Structure	Percent agreement	Mean CV	Percent agreement	Mean CV
		Between-readers		
Scales	20.0	32.5	22.4	27.8
Dorsal spines	58.5	11.9	51.8	12.5
Whole otoliths	59.5	9.1	82.2	3.3
Halved otoliths	77.2	3.2	96.2	0.7
		Versus halved otolith age (both readers combined)		
$Scales_1$	63.8	5.0	79.4	2.8
$Scales_2$	15.9	27.4	19.3	25.0
Dorsal $spines_1$	42.1	11.5	36.3	12.3
Dorsal $spines_2$	41.7	14.1	37.8	12.6
Whole $otolith_1$	61.6	4.8	83.8	2.3
Whole $otolith_2$	52.7	7.9	81.4	2.9

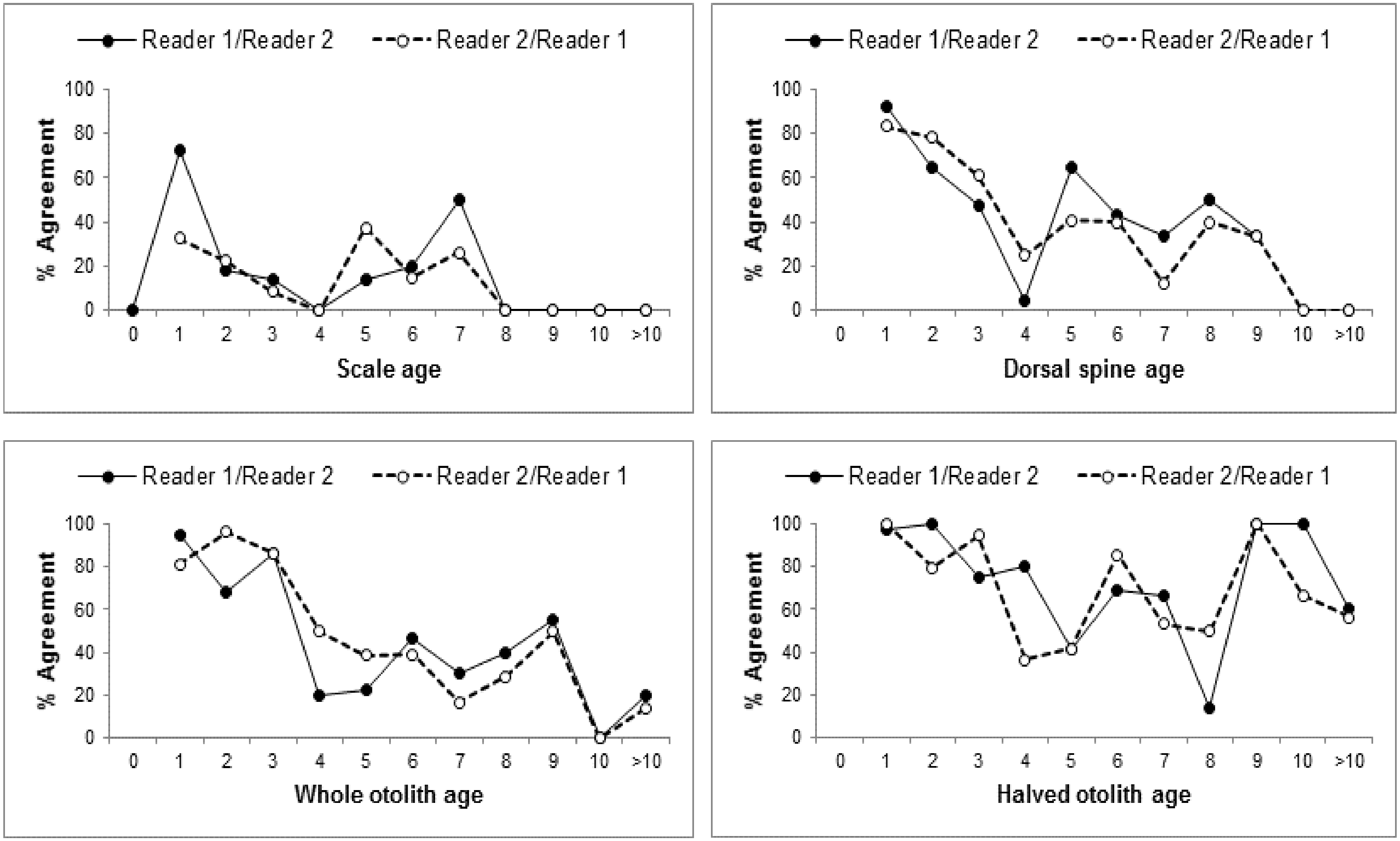
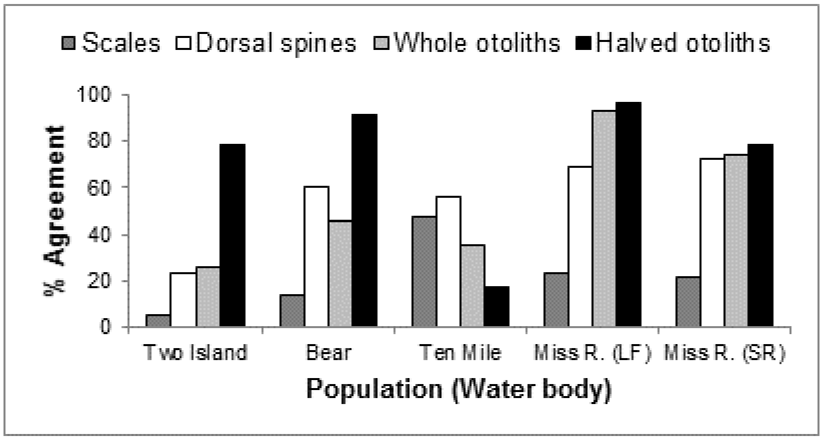


FIGURE 1. Mean percent agreement of scale age, dorsal spine age, whole otolith age, and halved otolith age estimates of Smallmouth Bass from Minnesota made by Reader 1 as a function of ages estimated by Reader 2 and by Reader 2 as a function of Reader 1.



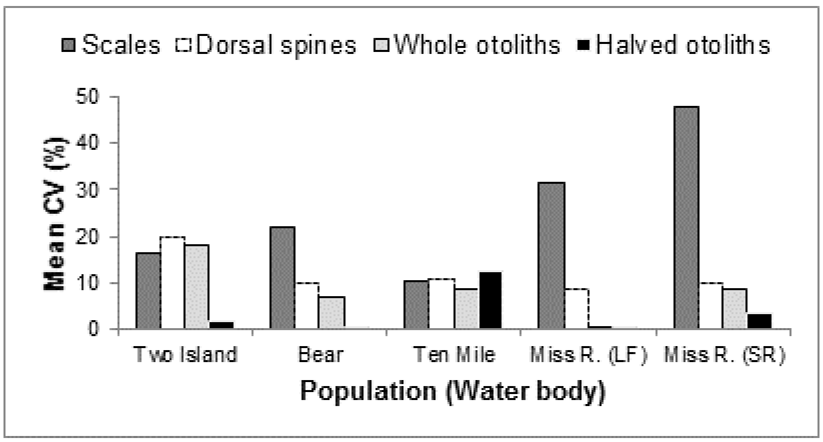


FIGURE 2. Percent between-reader agreement and mean coefficients of variation (CV) of Smallmouth Bass ages estimated with scales, dorsal spines, whole otoliths, and halved otoliths by population (water body).

Logistic linear mixed-effects modeling suggested that between-reader agreement was most influenced by mean structure age or the interaction between mean structure age and population. Between-reader agreement of scale ages, WO ages and HO ages was best explained by the mean structure age + population interaction (Table 3). However, mean DS age best explained between-reader agreement of DS age (Table 3).

Age-bias analyses suggested systematic bias occurred between scale ages, DS ages, and WO ages estimated by each reader, but age-bias did not occur between HO readers. From scale ages 1 through 8 Reader 1 usually counted fewer annuli on scales than Reader 2 but counted more annuli on older Smallmouth Bass (Table 4). Dorsal spine age estimated by Reader 1 usually exceeded DS ages estimated by Reader 2 at all DS ages, and WO age estimated by Reader 1 usually exceeded WO age estimated by Reader 2 after WO estimates exceeded age 3 (Table 4). Confidence limits of HO estimates usually overlapped among most HO ages, suggesting no age-bias between readers (Table 4).

Comparisons with halved otolith age

When compared to HO age estimates, WO age estimates, overall, were better than scale age estimates of younger (ages 1 to 3) Smallmouth Bass, but ages estimated with either structure were unreliable for bass older than age 4. Conversely, DS age estimates were unreliable for all ages except age 1, assuming HO age estimates were accurate.

For all Smallmouth Bass samples combined, WO age estimates agreed better with HO age estimates than scale ages estimated by Reader 2 and both readers of dorsal spines (Table 2). Similarly, mean CVs between WO age and HO age were lower than mean CVs between HO age and scale age estimated by reader 2 and HO age and DS ages estimated by either reader (Table 2). However, agreement between scale age estimates by Reader 1 and HO ages slightly exceeded agreement between both sets of WO ages and HO age (Table 2). Mean CV between scale age by Reader 1 and HO ages were similar to mean CV between WO age and HO age (Table 2).

TABLE 3. Akaike Information Criteria scores for logistic linear mixed effects models testing the effects of mean structure age, population, and mean structure age + population on age agreement between readers of scales, dorsal spines, whole otoliths, and halved otoliths from Smallmouth Bass and Largemouth Bass in Minnesota.

	Structure			
Independent variable	Scales	Dorsal spines	Whole otoliths	Halved otoliths
Smallmouth Bass				
Mean structure age	174	210	203	181
Population	182	237	221	174
Mean structure age + population	172	212	200	153
Largemouth Bass				
Mean structure age	403	505	339	129
Population	422	518	348	129
Mean structure age + population	399	503	317	130

TABLE 4. Mean (± 95% confidence limits) age estimated by one reader as a function of age estimated by a second reader of scales, dorsal spines, whole otoliths, and halved otoliths of Smallmouth Bass collected in Minnesota (R_1 denotes Reader 1; R_2 denotes Reader 2).

Age	Scales		Dorsal Spines		Whole otoliths		Halved otoliths	
	R_1R_2	R_2R_1	R_1R_2	R_2R_1	R_1R_2	R_2R_1	R_1R_2	R_2R_1
0	1.00±0.00							
1	1.41±0.35	1.18±0.24	1.08±0.08	1.17±0.10	1.12±0.20	1.19±0.11	1.02±0.05	1.00±0.00
2	1.55±0.28	2.96±0.50	1.98±0.20	2.00±0.16	1.84±0.18	1.96±0.08	2.00±0.00	2.15±0.15
3	3.14±0.81	4.26±0.92	3.65±0.65	2.72±0.29	3.18±0.22	2.86±0.16	2.75±0.19	3.05±0.11
4	2.75±0.72	5.89±2.44	5.48±0.51	3.00±1.30	5.10±0.63	3.50±0.92	3.80±0.56	4.64±0.34
5	4.43±0.74	4.38±0.88	5.59±0.55	4.44±0.32	6.27±0.46	4.08±0.67	4.42±0.32	5.58±0.33
6	6.07±1.01	6.35±0.98	7.14±1.13	5.00±0.55	6.60±0.35	5.44±0.35	5.77±0.21	6.14±0.17
7	7.08±1.14	7.83±0.73	9.00±3.30	4.50±1.18	8.30±1.26	5.83±0.49	6.83±0.37	7.33±0.35
8	6.20±1.17	10.75±5.72	9.00±2.25	6.40±2.08	8.60±1.88	7.14±1.24	7.14±0.35	7.5
9	9.25±7.28	7.33±1.44	11.33±6.25	7.33±5.17	9.67±1.15	8.20±1.06	9.00±0.00	9.00±0.00
10	8.70±1.79	7.00±2.48		6.50±0.92	12.33±7.59	9.00±4.97	10	10.33±1.44
11	10.33±4.49	10	12	7.67±3.79	10	8.5	10.75±0.80	11.00±0.00
12		10.20±1.04		12	13	9.5	12	12
13	7.5		14.00±4.30		15.33±1.58	10.67±5.17	14	
14	8	11.50±4.00		9	16	12.5	14.25±0.80	13.60±0.68
15	14			13		10		14
16						13.25±0.80	17	
17						13	17	17
18							18	19
19							18.5	18.5

Agreement between scale and HO ages, DS and HO ages, and WO and HO ages declined with increasing HO age estimates for both readers of each structure (Figure 3). Overall, ages estimated by scale Reader 1 usually equaled HO ages more than 80% of the time among HO ages 1 through 3, but agreement dropped below 80% after HO estimates exceeded age 3 (Figure 3). Conversely, agreement between scale ages estimated by Reader 2 and HO age did not exceed 80% at any HO age (Figure 3). Dorsal spine ages equaled HO ages over 90% of the time when HO age equaled 1 for all four reader-structure combinations, and agreement exceeded 80% for HO age 2 when dorsal spines were aged by Reader 2 (Figure 3). However, DS-HO age agreement dropped with increasing HO ages exceeding age 2 (Figure 3). Lastly, agreement between WO and HO ages exceeded 80% for all reader combinations for HO ages 1, 2, and 3, but WO-HO age agreement declined after HO ages reached age 4 and older (Figure 3).

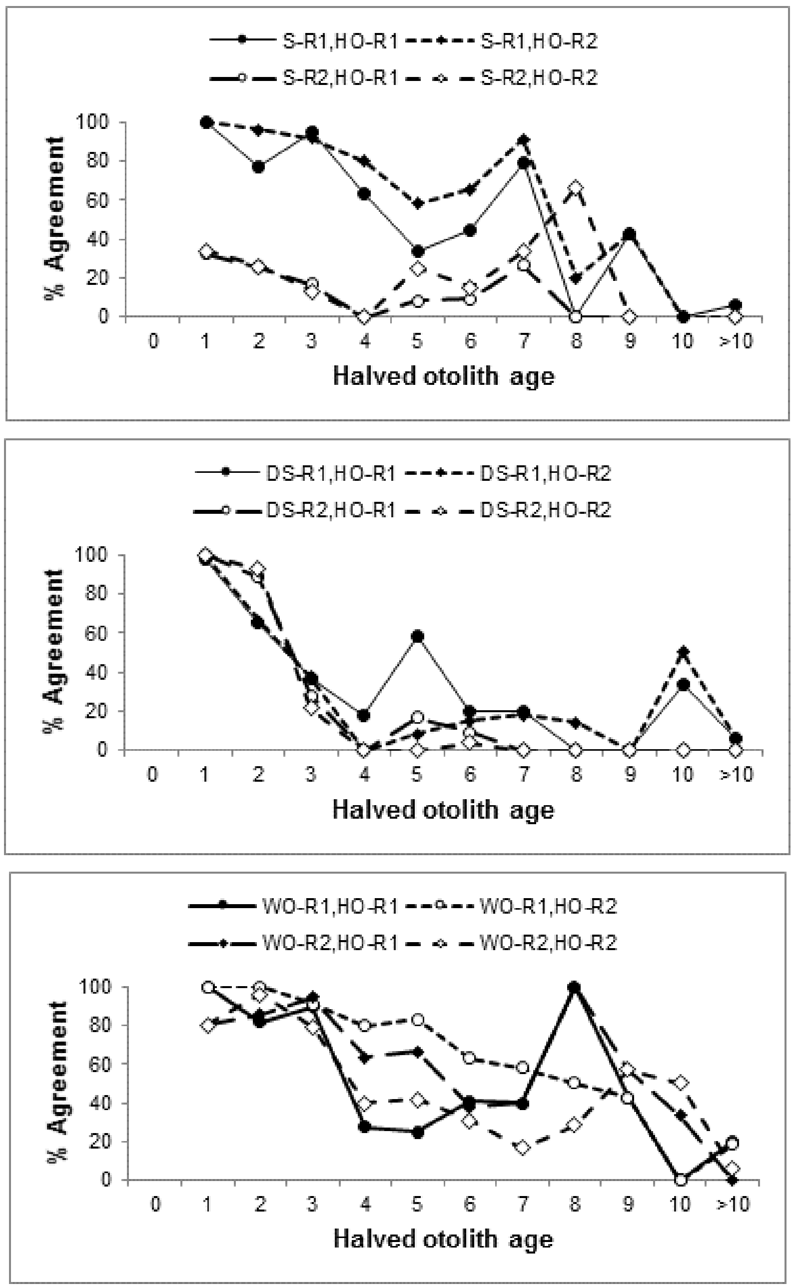
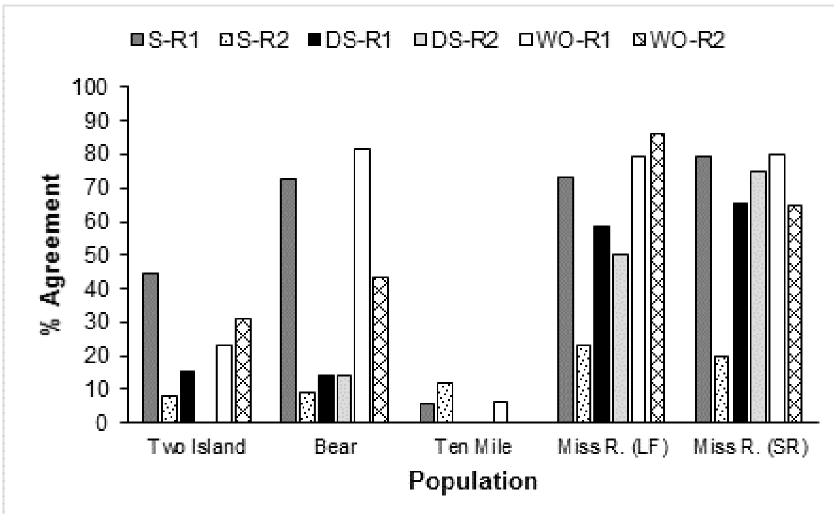


FIGURE 3. Percent agreement between scale (S) and halved otolith (HO) age, dorsal spine (DS) age and halved otolith age, and whole otolith (WO) and halved otolith age as a function of HO age of Smallmouth Bass in Minnesota estimated by two independent readers (R1 denotes Reader 1; R2 denotes Reader 2).

Precision also appeared to be affected by the population of Smallmouth Bass examined. Agreement between scale age estimates made by Reader 1 and HO ages ranged from 6 to 80% among populations, and agreement equaled 80% in only one of the five populations (Figure 4). Conversely, agreement between scale ages by Reader 2 and HO ages ranged from 9 to 23% (Figure 4). Mean CV between ages estimated by Reader 1 and both estimates of HO ages ranged from 3 to 11% among populations, whereas mean CV between scale ages by Reader 2 and HO ages ranged from 15 to 37% (Figure 4). Agreement between DS ages by Reader 1 and HO age and by Reader 2 and HO age ranged from 0 to 75% among populations, and mean CV ranged from 7 to 26% (Figure 4). Agreement between WO ages estimated by Reader 1 and HO age ranged from 6 to 82% among populations and equaled or exceeded 80% in two of the five populations (Figure 4). For Reader 2 of whole otoliths, WO-HO age agreement ranged from 0 to 86% and exceeded 80% in only one population (Figure 4). Mean CV between WO and HO ages ranged from 2 to 10% (Reader 1) and 1 to 12% (Reader 2) among populations (Figure 4).



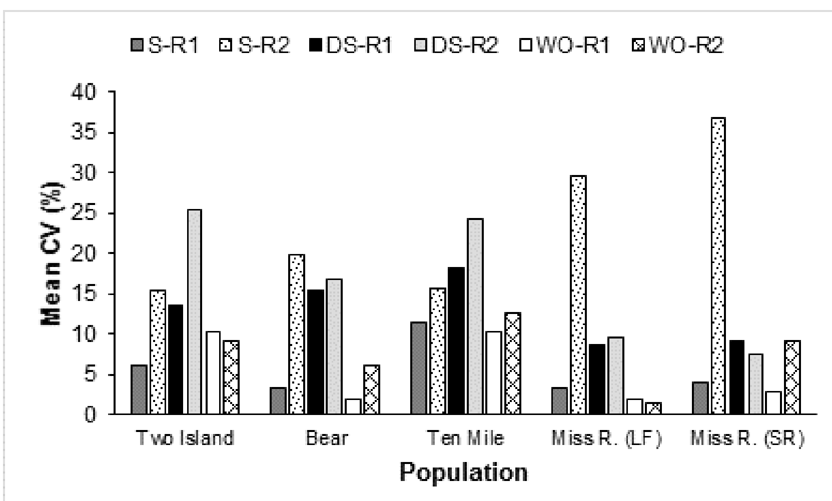


FIGURE 4. Percent agreement and mean coefficients of variation (CV) between scale (S) ages by two readers (R1 denotes reader 1; R2 denotes reader 2) and halved otolith age, dorsal spine (DS) ages by two readers and halved otolith age, and whole otolith (WO) age by two readers and halved otolith age of Smallmouth Bass as a function of population.

Logistic linear mixed-effects modeling suggested that between-structure agreement was most affected by mean HO age or the mean HO age + population interaction. Agreement between scale age by Reader 2 and HO age and between DS age by each reader and HO age was most affected by mean HO age (Table 5). Agreement between scale age by Reader 1 and HO age and between WO age by each reader and HO age was best explained by the mean HO age + population interaction (Table 5).

Age-bias plots showed varying degrees of between-structure bias among structures and readers. Scale age estimates made by Reader 1 appeared unbiased among HO ages 1 through 7, then scale ages declined with increasing HO age (Figure 5). Conversely, scale ages estimated by Reader 2 showed positive bias with respect to HO ages 1 through 4, showed no bias from HO ages 5 through 8, and then showed negative bias at HO ages exceeding age 8 (Figure 5). Dorsal spine ages by both readers indicated negative bias after HO ages exceeded age 1 (Figure 5). Whole otolith age estimated by Reader 1 appeared unbiased from HO ages 1 through 9, and then became negatively biased with increasing HO age exceeding 9 (Figure 5). Conversely, WO ages estimated by Reader 2 were unbiased among HO ages 1 through 4, but showed negative bias after HO ages exceeded age 4 (Figure 5).

TABLE 5. Akaike Information Criteria scores for logistic linear mixed-effects models testing the effects of population, mean halved otolith (HO) age, and the mean HO age + population interaction on age agreement between selected combinations of scale (S) readers and HO readers, dorsal spine (DS) readers and HO readers, and whole otolith (WO) readers and HO readers for Smallmouth Bass and Largemouth Bass in Minnesota ($_1$ denotes Reader 1; $_2$ denotes Reader 2).

	Structure combination					
Independent variable	$S_1HO_1HO_2$	$S_2HO_1HO_2$	$DS_1HO_1HO_2$	$DS_2HO_1HO_2$	$WO_1HO_1HO_2$	$WO_2HO_1HO_2$
Smallmouth Bass						
Mean HO age	156	139	154	75	170	190
Population	221	164	212	172	202	230
Mean HO age + population	141	141	154	77	152	172
Largemouth Bass						
Mean HO age	316	389	449	410	289	320
Population	396	392	485	473	342	338
Mean HO age + population	318	383	439	389	288	281

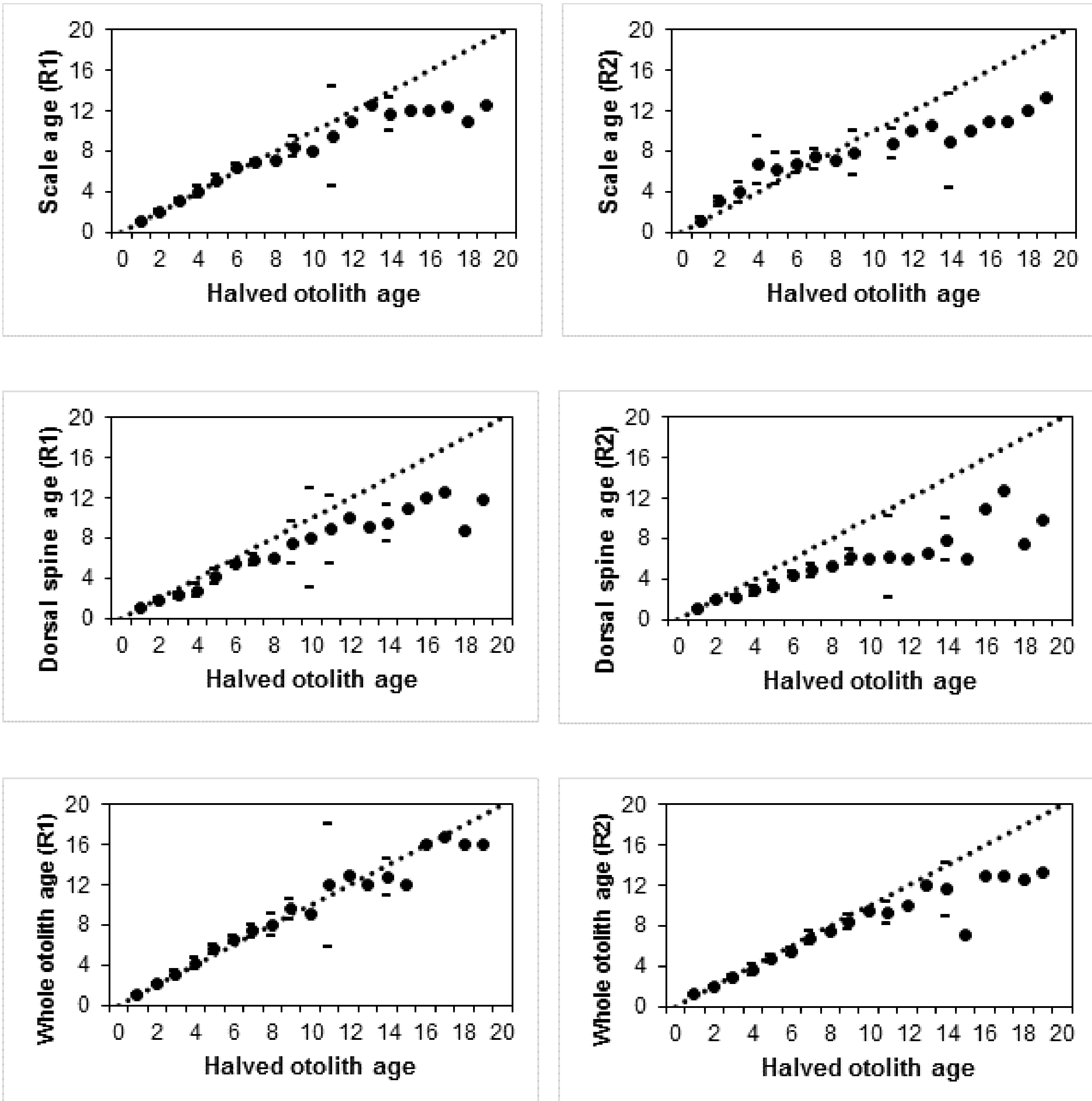


FIGURE 5. Age-bias plots of mean scale age by Reader 1 (R1), mean scale age by Reader 2 (R2), mean dorsal spine age by Reader 1, mean dorsal spine age by Reader 2, mean whole otolith age by Reader 1 and mean whole otolith age by Reader 2 as a function of halved otolith age of Smallmouth Bass from Minnesota (solid circles denote mean; horizontal lines above and below solid circles denote 95% confidence limits).

Largemouth Bass

Between-reader analyses of aging structures

Between-reader analyses of aging structures from Largemouth Bass also suggested that HO age estimates are the most precise and least biased compared to WO age, DS age, and scale age estimates. Between-reader agreement of HO age estimates from all Largemouth Bass examined in this study exceeded 96% and mean CV was less than one percent (Table 2). Agreement between readers of whole otolith exceeded 80% with mean CV being below 4% (Table 2). Conversely, between-reader agreement of DS age and scale ages were below 60%, and mean CV exceeded 10% (Table 2).

Between-reader precision of scale ages, DS ages, and WO ages declined with increasing age or differed substantially among populations of Largemouth Bass, but precision of HO ages was not affected by either variable. Age agreement between readers of scales declined with increasing scale age, but did not exceed 80% for any scale age (Figure 6). Between-reader agreement of scale ages ranged from 0 to 38% among populations, and mean CV ranged from 17 to 62% among populations (Figure 7).

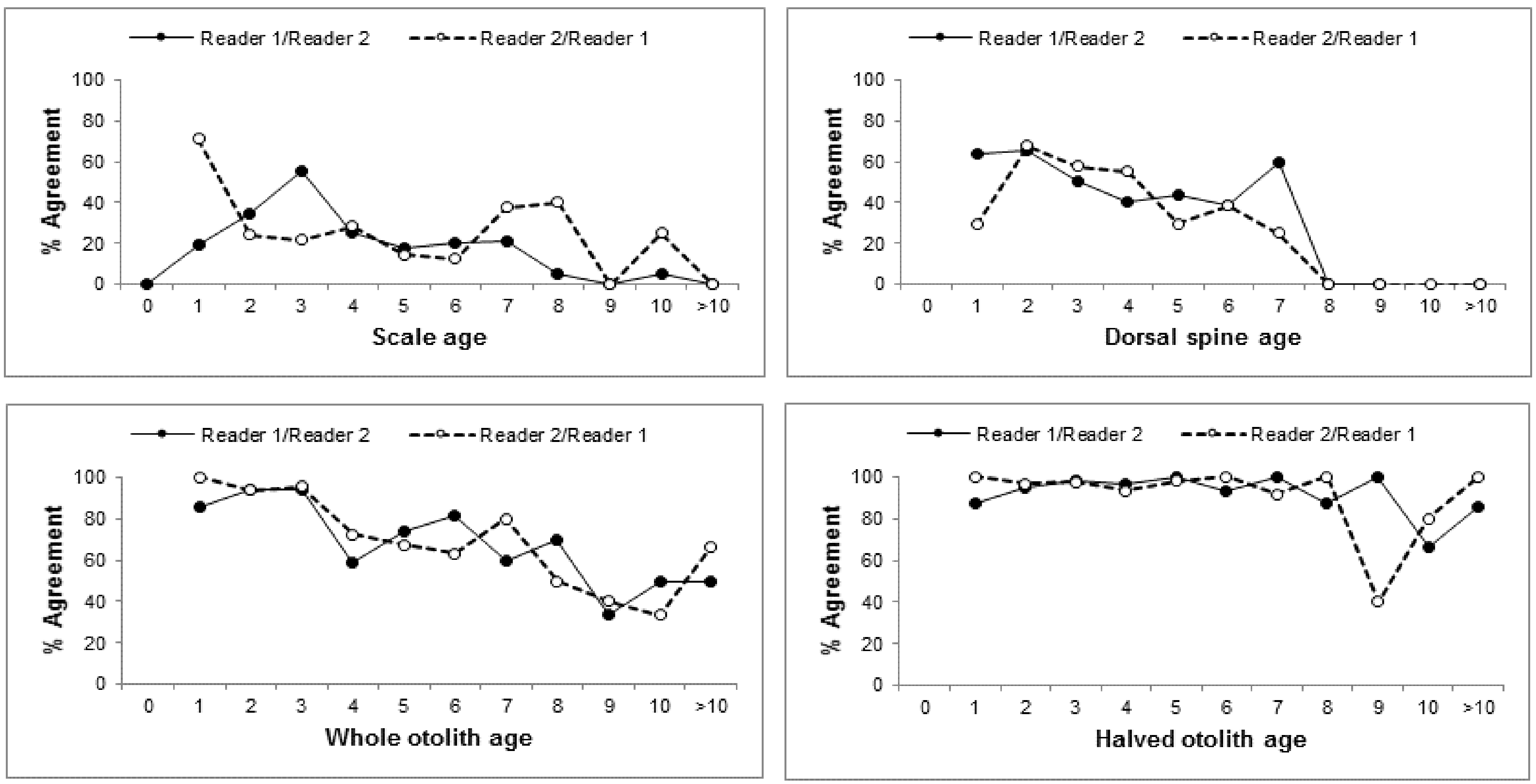


FIGURE 6. Mean percent agreement of scale age, dorsal spine age, whole otolith age, and halved otolith age estimates of Largemouth Bass from Minnesota made by Reader 1 as a function of ages estimated by Reader 2 and by Reader 2 as a function of Reader 1.

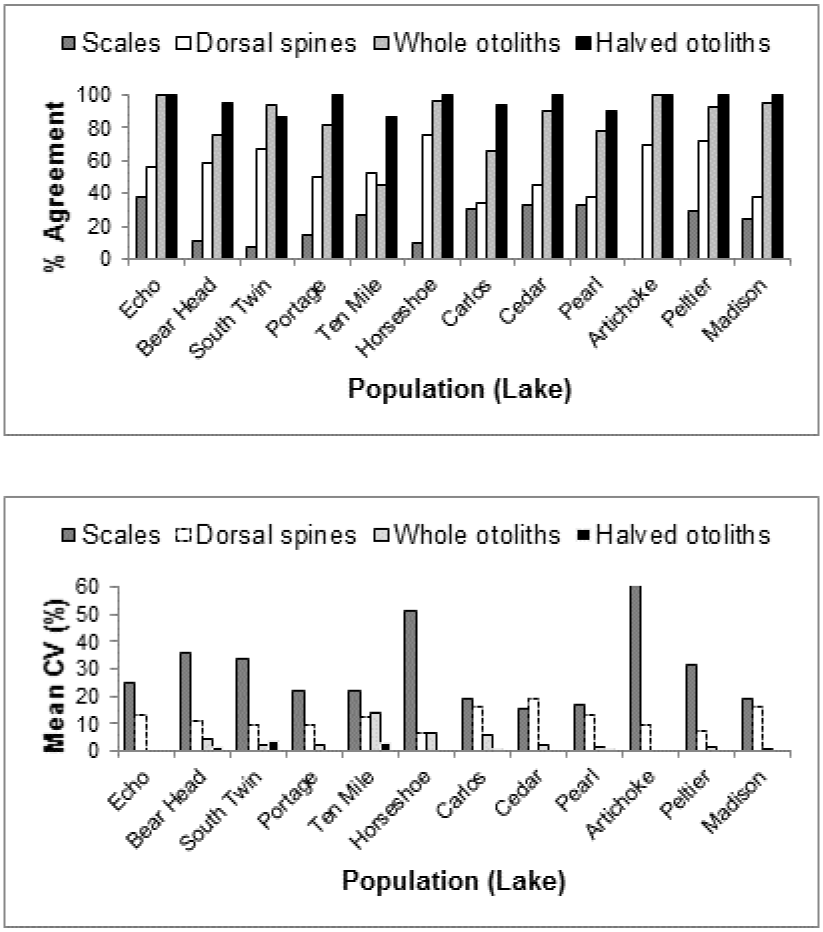


FIGURE 7. Percent between-reader agreement and mean coefficients of variation (CV) of Largemouth Bass ages estimated with scales, dorsal spines, whole otoliths, and halved otoliths by population (or lake).

Between-reader agreement of DS age declined with increasing DS age, and also did not exceed 80% among any DS age (Figure 6). Between-reader agreement of DS ages ranged from 34 to 76% and mean CV ranged from 6 to 19% among populations (Figure 7). For WO ages, between-reader agreement exceeded 80% among WO ages 1 through 3, but agreement declined with increasing WO age after WO age exceeded age 3 (Figure 6). Between-reader agreement of WO ages ranged from 46 to 100% among populations, and agreement exceeded 80% in eight of the 12 populations (Figure 7). Mean CV of WO ages ranged from 0 to 14% among populations (Figure 7). Between-reader agreement of HO age exceeded 80% for all HO ages except HO ages 9 and 10 (Figure 6). Agreement between HO ages ranged from 86 to 100% and mean CV ranged from 0 to 4% among the 12 populations (Figure 7). Logistic linear mixed-effects modeling suggested that between-reader agreement of scale age, DS age, and WO age was affected by the appropriate mean structure age + population interaction (Table 3). Conversely, mean HO age, population or the HO age + population interaction did not appear to affect between-reader agreement of HO age (Table 4).

Age-bias analyses suggested clear aging bias between the two scale readers, but no clear reader bias occurred for DS age, WO age, or HO age estimates (Table 6). Reader 2 counted more annuli on scales than Reader 1 after scale ages exceeded age 2 (Table 6).

TABLE 6. Mean (± 95% confidence limits) age estimated by one reader as a function of age estimated by a second reader of scales, dorsal spines, whole otoliths, and halved otoliths of Largemouth Bass collected in Minnesota (R_1 denotes Reader 1; R_2 denotes Reader 2).

	Scales		Dorsal Spines		Whole otoliths		Halved otoliths	
Age	R_1R_2	R_2R_1	R_1R_2	R_2R_1	R_1R_2	R_2R_1	R_1R_2	R_2R_1
0	1							
1	2.00±0.26	1.00±0.53	1.36±0.34	1.75±0.22	1.14±0.35	1.00±0.00	1.12±0.30	1.00±0.00
2	2.65±0.19	2.58±0.39	2.13±0.12	2.26±0.10	2.11±0.11	2.03±0.06	2.05±0.05	2.00±0.05
3	2.70±0.17	4.62±0.38	2.86±0.17	2.90±0.16	3.03±0.05	3.04±0.04	3.01±0.02	2.99±0.02
4	3.38±0.24	5.29±0.68	4.30±0.23	3.92±0.24	4.24±0.20	3.85±0.22	3.97±0.07	3.93±0.10
5	3.64±0.33	7.43±0.67	5.22±0.33	4.47±0.27	5.10±0.21	4.75±0.16	5.00±0.00	5.02±0.03
6	4.03±0.46	8.25±0.55	6.00±0.48	5.24±0.33	6.06±0.22	5.93±0.22	6.02±0.08	6.00±0.00
7	5.24±0.47	8.75±0.87	6.60±2.72	6.00±0.72	6.70±0.27	6.87±0.35	7.00±0.00	6.92±0.11
8	4.85±0.54	8.40±1.42	7.33±3.79	6	8.10±0.41	7.64±0.70	8.12±0.30	8.00±0.00
9	4.97±0.70	12.14±1.72	10	8.00±3.44	8.67±0.86	8.80±1.04	9	9.20±1.04
10	6.00±0.86	12.00±4.68		10.5	9.5	10.00±2.48	9.67±0.54	10.20±0.56
11	5.67±0.91		9		10.5	11	10.75±0.80	11.00±0.00
12	6.80±1.84	14	10		12	12		
13			14				13	13
14	9.17±2.04			13	15			
15	8.5					14		
17							17	17
21							21	21

Comparisons with halved otolith age

Assuming that HO ages are accurate, WO ages appear reliable for estimating age of younger (ages 1 to 4) Largemouth Bass, scale ages might be reliable for estimating age of younger Largemouth Bass depending on reader, but DS age estimates appear unreliable. The best overall between-structure precision (> 80% agreement; < 3% mean CV) occurred between WO and HO age estimates (Table 2). Precision between scale age by Reader 1 and HO age was nearly as good as that found for WO-HO comparisons (Table 2). Conversely, precision between DS and HO age and between scale age by Reader 2 and HO age was poor (< 40% agreement; > 12% mean CV) (Table 2).

Agreement between scale and HO age, DS and HO age, and WO and HO age of Largemouth Bass generally decreased with increasing HO age, but patterns in these declines differed among structures. Agreement between scale ages by Reader 1 and HO age exceeded 80% for HO ages 1 through 3, but scale age declined with increasing HO age exceeding age 3 (Figure 8). However, agreement between ages by scale Reader 2 and HO age did not exceed 80% for any HO age, but scale age also declined with increasing HO age (Figure 8). At HO age 1, all DS ages estimated by both readers equaled age 1, but agreement dropped below 80% by as early as HO age 2 and declined with increasing HO ages exceeding age 2 (Figure 8). Whole otolith ages estimated by both readers usually exceeded 80% agreement with HO ages 1 through 4 (Figure 8). Agreement in all combinations of WO and HO age estimates declined between HO ages 5 through 8, and then dropped substantially after HO otolith ages exceeded age 8 (Figure 8).

Between-structure agreement and mean CV also differed among populations of Largemouth Bass. Agreement between scale ages by Reader 1 and HO age ranged from 48 to 100% among 12 populations, and exceeded 80% in seven populations (Figure 9). Mean CV between scale age by Reader 1 and HO age ranged from 0 to 7% (Figure 9). Conversely, agreement between scale ages estimated by Reader 2 and HO age ranged from 0 to 36% and mean CV ranged from 13 to 61% among populations (Figure 9). Agreement between DS and HO ages ranged from 17 to 80% (Reader 1) and 17 to 79% (Reader 2) among populations, and mean CV ranged from 4 to 17% (Reader 1) and 5 to 18% (Reader 2) (Figure 9). Agreement between WO and HO ages ranged from 45 to 100% among populations for both WO readers combined, and mean CV ranged from 0 to 12% (Figure 9). Agreement between WO age by Reader 1 and HO age exceeded 80% in eight populations and exceeded 80% in six populations by Reader 2 (Figure 9).

Logistic linear mixed-effects modeling suggested that mean HO age or the mean HO age + population interaction affected agreement between scale age and HO age, DS age and HO age, and WO age and HO age. Agreement between scale ages estimated by Reader 1 and HO age and between WO age by Reader 1 and HO age was best explained by variation in mean HO age (Table 5). However, agreement between scale ages by Reader 2 and HO age, DS age by each reader and HO age, and WO age by Reader 2 and HO age were best explained by the HO age + population interaction (Table 5).

Age-bias plots showed variable biases between scale age and HO age, DS age and HO age, and WO age and HO age. Scale ages estimated by Reader 1 showed no bias among HO ages 1 through 6, then showed negative bias after HO age exceeded age 6 (Figure 10). Conversely, scale ages estimated by Reader 2 showed positive bias among HO ages 2 through 7, no bias between HO ages 8 through 13, and negative bias when compared to the oldest HO ages (Figure 10). Dorsal spine ages were unbiased at HO age 1, but negatively biased after HO ages exceeded 1 or 2 (Figure 10). Whole otolith ages were unbiased among HO ages 1 through 4, but WO ages became negatively biased with increasing HO age at HO age 5 (Figure 10).

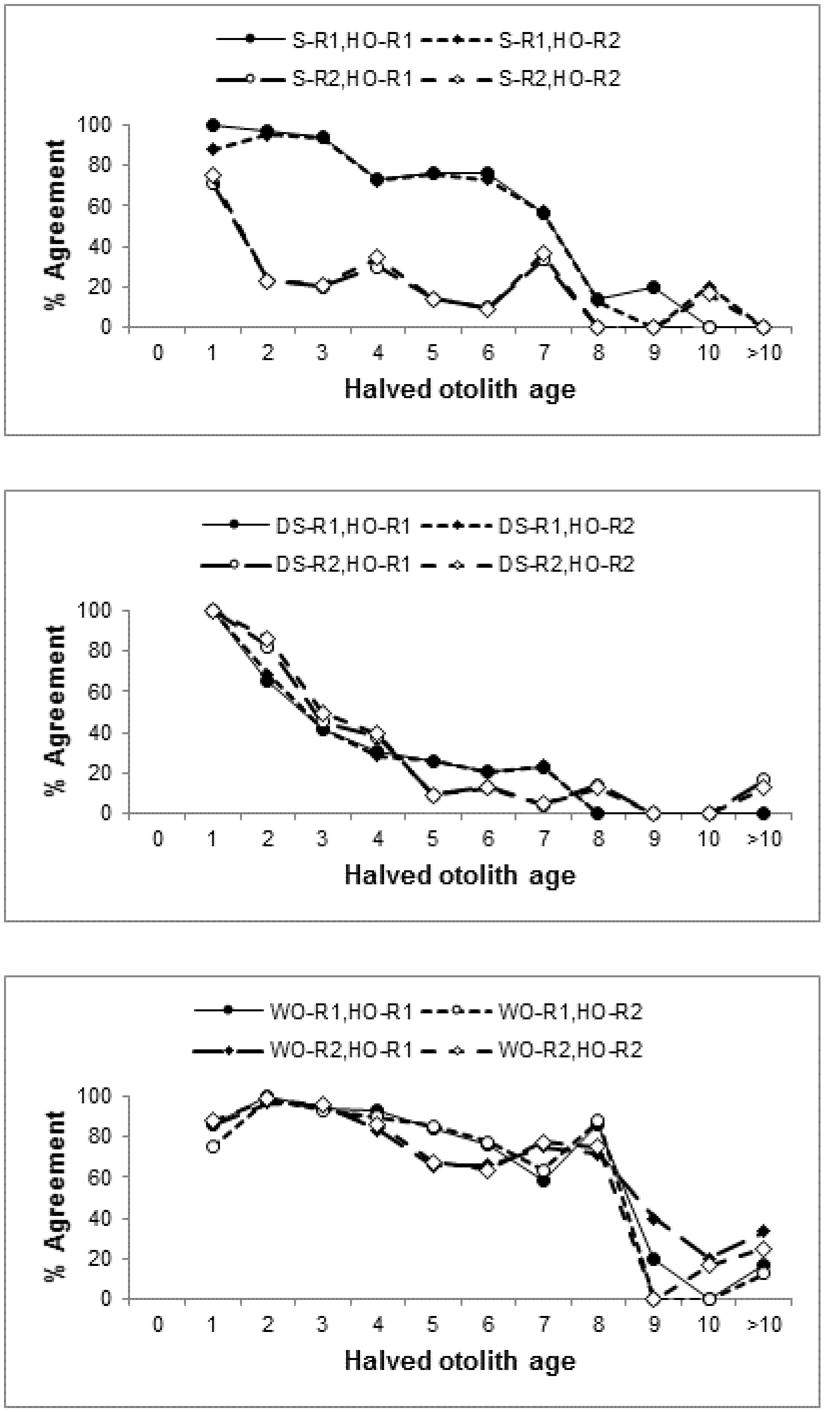
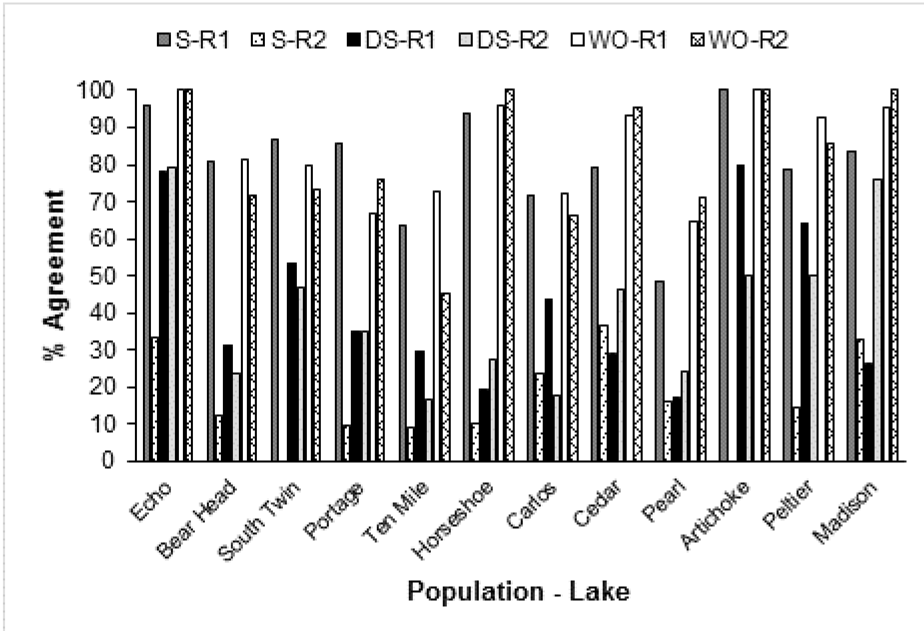


FIGURE 8. Percent agreement between scale (S) and halved otolith (HO) age, dorsal spine (DS) age and HO age, and whole otolith (WO) and HO age as a function of HO age of Largemouth Bass in Minnesota estimated by two independent readers (R1 denotes Reader 1; R2 denotes Reader 2).



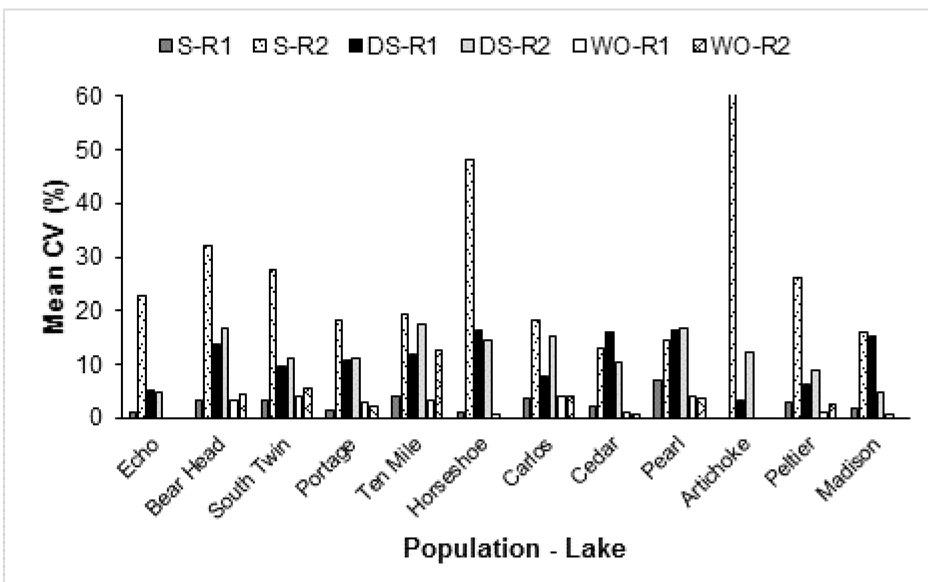


FIGURE 9. Percent agreement and mean coefficients of variation (CV) between scale (S) ages by two readers (R1 denotes reader 1; R2 denotes reader 2) and halved otolith age, dorsal spine (DS) ages by two readers and halved otolith age, and whole otolith (WO) age by two readers and halved otolith age of Largemouth Bass as a function of population (lake).

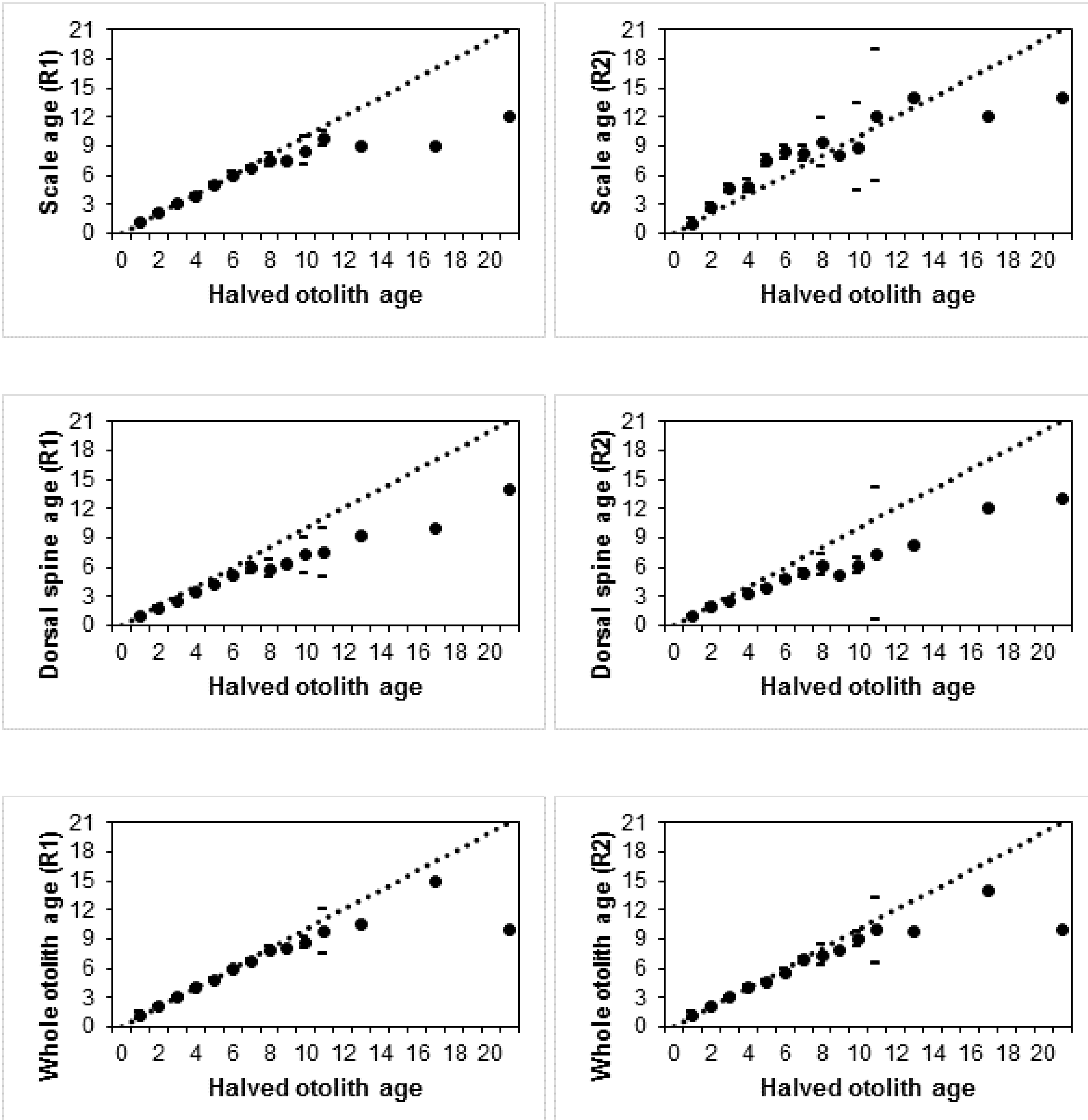


FIGURE 10. Age bias plots of mean scale age by Readers 1 (R1) and 2 (R2), mean dorsal spine age by Readers 1 and 2, and mean whole otolith age by Readers 1 and 2 as a function of halved otolith age of Largemouth Bass from Minnesota lakes (solid circles denote mean; horizontal lines above and below solid circles denote 95% confidence limits).

DISCUSSION

Our study showed that scales, dorsal spines, and whole otoliths have limited or no applicability for providing age estimates of Smallmouth Bass or Largemouth Bass in Minnesota. At best, WO age estimates appear reliable for estimating age of Smallmouth Bass from 1 to 3 years of age and Largemouth Bass from 1 to 4 years of age for most populations in Minnesota. Competent scale readers could provide reliable age estimates of most populations of both bass species ranging from ages 1 to 3. Whole otoliths and scales examined by competent readers could also provide reliable estimates of ages 4 through 7 in some bass populations (Maraldo and MacCrimmon 1979; Besler 2001; Long and Fisher 2001; Maceina and Sammons 2006). However, our study suggests that MNDNR staff would need to conduct population-specific structure comparisons that include either known-age or sectioned/HO ages in order to support their scale or WO age estimates of bass older than age 4. Lastly, our study supports conclusions made by others that DS age estimates provide unreliable estimates of age for either bass species (Maraldo and MacCrimmon 1979; Sotola et al. 2014).

The assumption that HO ages are accurate appears reasonable for most populations of Smallmouth Bass and Largemouth Bass in this study; overall between-reader precision in this study was as good as or better than observed elsewhere even though we observed older individuals in this study. Between- or among-reader mean CV of sectioned or HO ages ranged from 7 to 13% between populations of Smallmouth Bass in Oklahoma and New York (Long and Fisher 2001; Sotola et al. 2014). For Largemouth Bass, between- or among-reader CV of sectioned or HO ages ranged from 0.4 to 15% among populations in North Carolina, Oklahoma, New York, and South Africa (Besler 2001, Long and Fisher 2001, Taylor and Weyl 2012; Sotola et al. 2014). These were similar to mean CV of age averaging 4% (range = 0.2 to 13%) among the five Smallmouth Bass populations, and mean CV averaging 0.8% (range = 0 to 4%) among the 12 Largemouth Bass populations in this study. Maximum HO ages in this study (19 for Smallmouth Bass; 21 for Largemouth Bass) exceeded those in the other studies (5 to 14 for Smallmouth Bass; 7 to 19 for Largemouth Bass) (Long and Fisher 2001; Maceina and Sammons 2006; Taylor and Weyl 2012; Sotola et al. 2014). Therefore, overall conclusions in this study about the merits of scales, dorsal spines, and whole otoliths as aging structures would likely be similar to those made if structures came from known-aged bass.

Between-reader precision of WO ages of both bass species in this study appeared similar to worse than found in other studies, and some of these differences could be caused by different processing methods, thickness of otoliths, and quality of otoliths. Mean CV of WO age for an Oklahoma population of Smallmouth Bass equaled 9%, and mean CV of WO age ranged from 0.9 (North Carolina) to 12% (Oklahoma) for populations of Largemouth Bass (Besler 2001; Long and Fisher 2001). Mean CV of WO age of Smallmouth Bass averaged 9% (range = 0.6 to 18%) and mean CV of WO age of Largemouth Bass averaged 3% (range = 0 to 14%) among populations in this study. Maceina and Sammons (2006) reported between-reader agreement exceeding 90% for WO ages 1 through 6 Smallmouth Bass and Largemouth Bass in New York. Percent agreements of WO age of Smallmouth Bass averaged 55% among five populations and averaged 84% among Largemouth Bass populations in this study. Maceina and Sammons (2006) clarified otoliths by soaking them in a glycerin or ethanol/glycerin solution for at least a month, and this process likely contributed to the very high between-reader agreements they reported. Conversely, whole otoliths in this study, the Oklahoma study, and the North Carolina study were stored dry before estimating ages (Besler 2001; Long and Fisher 2001). Without clarification by glycerin, whole otoliths of older bass in these studies were probably too thick for readers to effectively find innermost annuli. Lastly, we also observed that one of the pair of otoliths from some (we did not determine prevalence) Smallmouth Bass were deformed, whereas the other appeared normal. Even though appearing normal, the other otolith from these pairs may still have been difficult to interpret. All otoliths from Largemouth Bass appeared normal.

The lower precision of HO and WO estimates observed for Smallmouth Bass (compared to precision of HO and WO estimates in Largemouth Bass) in this study could be affected by uncertain interpretation of annulus appearance on the otolith edge coupled with the age of bass. Three of the five Smallmouth Bass populations were sampled in July, whereas only two of the 12 Largemouth Bass populations were sampled during July. The other samples were collected in May, early June, late August and September. Furthermore, HO ages of Smallmouth Bass in this study averaged 4.7 years compared to 4.1 years for Largemouth Bass. Although unknown for either species of bass, annulus appearance on otoliths of older Yellow Perch *Perca flavescens* in South Dakota oftentimes was not observed until August (Blackwell and Kaufman 2012). Thus, readers probably had difficulty determining if the otolith edge was an annulus because annulus appearance may not have been complete in all bass collected in July. The same two people provided all HO estimates, but the second reader providing WO estimates differed between species. However, all WO and HO readers in this study should be viewed as competent with these two aging methods.

Precision between readers of dorsal spines of both bass species appeared similar to precision found in the other study reporting these data. Sotola et al. (2014) reported among-reader mean CV of 9% and 37% agreement for DS age in a Smallmouth Bass population and mean CV of 13% and percent agreement of 31% of DS age in a Largemouth Bass population in New York. These were within the ranges of mean CV (9 to 20% for Smallmouth Bass; 6 to 19% for Largemouth Bass) and percent agreement (23 to 72% for Smallmouth Bass; 38 to 76% for Largemouth Bass) among populations in our study.

Low precision and negative bias in DS estimates of both bass species appears related to a conspicuous lumen in the center of the dorsal spines, crowding of annuli at the edge of spines, and reader inexperience. Dorsal spines of both bass species possessed lumens that partially or fully obscured at least one of the earliest annuli. Isermann et al. (2010) also observed lumens on dorsal spines of another centrarchid (Black Crappie *Pomoxis nigromaculatus*), and they concluded that these lumens obscured annuli and caused negatively biased age estimates when compared to otolith ages. Similar to that found in most structures, annuli on dorsal spines also crowd near the spine edge in older bass and become less distinguishable, and this caused additional negative bias in DS estimates. Lastly, both readers were independently self-taught and equally inexperienced in estimating age of bass via dorsal spines. Because marks were usually clear on dorsal spines, we hypothesized that between-reader precision of DS ages would improve with increased experience and consensus aging, but DS estimates would remain negatively biased because of the lumen and crowding of annuli near the edge. Our DS age estimates were made from unsectioned dorsal spines whereas Maraldo and MacCrimmon (1979) and Sotola et al. (2014) estimated age from cross-sections of dorsal spines. However, we cannot conclude if precision was affected by different processing methods.

Between-reader precision of scale estimates of both species in this study appeared poor compared to those reported in other studies. Between- or among-reader mean CV of scale ages ranged from 12 to 13% and percent agreement ranged from 17 to 67% for Smallmouth Bass populations in Oklahoma and New York (Long and Fisher 2001; Maceina and Sammons 2006; Sotola et al. 2014). Similarly, mean between- or among-reader CV of scale age ranged from 5 to 15% and percent agreement ranged from 20 to 57% for Largemouth Bass populations in Oklahoma, North Carolina, and New York (Besler 2001; Fisher and Long 2001; Maceina and Sammons 2006; Sotola et al. 2014). In this study, mean CV of scale age averaged 26% (range = 10 to 48%) and percent agreement averaged 22% (range = 14 to 47%) among populations of Smallmouth Bass, and mean CV averaged 29% (range = 17 to 62%) and agreement averaged 21% (range = 0 to 38%) among populations of Largemouth Bass.

Low precision and fluctuating bias in scale estimates were linked with different levels of reader experience, presence of false annuli, and crowding of annuli near the scale edge. Both scale readers possessed considerable practical experience estimating scale ages of several species, but one reader had substantially more experience estimating scale ages of bass than the other. The less experienced reader likely counted as annuli more false annuli than the other reader, resulting in positive bias in scale age estimates. This bias was common among age structure studies of bass that includes scales. Positive bias in scale age estimates occurred in populations of younger (< 9 years known-age or sectioned-HO-age) Smallmouth Bass in

Oklahoma and Largemouth Bass in Texas, North Carolina, and New York (Prentice and Whiteside 1974; Besler 2001; Long and Fisher 2001; Maceina and Sammons 2006). Conversely, positive bias in scale ages was not detected in other populations of bass in Alabama, Oklahoma, or New York (Prather 1966; Long and Fisher 2001; Maceina and Sammons 2006), suggesting that false annuli do not always form on scales or that scale readers distinguished them from true annuli. Negative bias in scale ages in the oldest bass of both species occurred in several populations of bass (Besler 2001; Maceina and Sammons 2006; Taylor and Weyl 2012), and is likely caused by crowding of annuli on the scale edge. We made scale impressions which is better than glass-mounting for revealing annuli on scales (Gürsoy et al. 2005; Ross et al. 2005); thus, poor precision in scale age estimates in this study is not linked to methodology.

MANAGEMENT IMPLICATIONS

This study clearly demonstrated that ages of Smallmouth Bass and Largemouth Bass estimated with scales, dorsal spines, and whole otoliths cannot be universally used as surrogates for ages estimated with halved otoliths. Thus, MNDNR staff should rely on HO age estimates (or estimates made with sectioned otoliths) if accurate and precise estimates of age are needed for all lengths of bass. If readers are properly trained, ages estimated from scales and whole otoliths will be useful if bass are less than ages 4 or 5, and will be useful for ages up to ages 6 or 7 in some populations of bass if age structure comparison tests demonstrate that scale and WO age estimates agree with HO age estimates. Lastly, dorsal spines should not be used as aging structures for either species of bass.

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