Minnesota Department of Natural Resources
Investigational Report 573, December 2020

Influence of age, population, total length, sex, and sample month on precision and bias of scale and whole otolith age estimates of Black Crappie in Minnesota

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Abstract - We determined precision and bias of age estimates of Black Crappie *Pomoxis nigromaculatus* from 49 Minnesota populations using annuli counts on scales and whole otoliths, and then determined the effects of age, population, gill net catch per lift (CPUE; an index of Black Crappie density) sample month, sex, latitude, longitude, lake size, and lake depth on precision and bias. We used for comparison the crack-burn method to count annuli on transverse views of otoliths (CB otolith), and assumed that this method provided the most accurate age estimates of Black Crappie. Overall, scale age estimates were less precise, agreed less with CB otolith age, and were more negatively biased with respect to CB otolith age than whole otolith age estimates. For both scales and whole otoliths, between-reader agreement, agreement with CB otolith age, and age-bias as a function of CB otolith age declined with increasing age and differed among populations. Gill net CPUE, longitude, lake size and depth, sampling season, and sex did not explain differences in precision and age-bias among populations; however, scale age estimates improved with increasing latitude within the state. Scale age estimates were useful from ages 0 through 4, whereas whole otolith age estimates were useful from ages 0 through 5. However, age of older Black Crappie should be estimated with annuli counts on transverse views of otoliths. While whole otoliths provided better age estimations than scales for most populations, scales performed well enough to remain a viable option for some populations if readers are properly trained.

This project was funded in part by the Federal Aid in Sport Fish Restoration (Dingell-Johnson) Program.
Completion Report, Study 638, D-J Project F-26-R Minnesota.

INTRODUCTION

Staff from the Minnesota Department of Natural Resources (MNDNR) often estimate age of Black Crappie *Pomoxis nigromaculatus* via annuli counts on scales or whole views of sagittal otoliths (whole otoliths) (McInerny et al. 2017); however, age precision or age-bias of either structure from Minnesota populations have not been evaluated. Because crappies (Black Crappie and the much less common White Crappie *P. annularis*) rank second in preference among anglers in Minnesota (Schroeder 2012), precise and unbiased estimates of age will often be needed for effective management of Black Crappie fisheries throughout the state.

Existing studies clearly show that age estimates from whole otoliths of Black Crappie are reliable, but age estimates from scales show inconsistent reliability. Whole otolith age estimates made by multiple readers equaled known-age (ages 1 through 5) Black Crappie from Kentucky hatchery ponds, and whole otolith age estimates equaled ages from otolith cross-sections from three Florida populations of Black Crappie (Schramm and Doerzbacker 1985; Crumpton et al. 1988; Ross et al. 2005). Conversely, agreement between scale age among two to four readers and known-age Black Crappie raised in those Kentucky hatchery ponds averaged 78-80% (Ross et al. 2005). Additionally, agreement between scale age and otolith (whole or sectioned) age ranged from 59-100% among five populations in North/South Carolina, South Dakota, and Minnesota (McInerny 1989; Kruse et al. 1993; Isermann et al. 2010c).

Differences in experience or competency among scale readers could explain differences between scale age and otolith age or scale age and known age Black Crappies among studies, but whole otolith readers appear similarly competent among studies. Kruse et al. (1993) used experienced readers to estimate scale age of Black Crappies in South Dakota, contributing to 97% age agreement among three readers. Conversely, scale age agreement among three readers equaled 33-36% for two Minnesota populations of Black Crappie. However, these MNDNR readers had only 2-5 years of practical experience, and they received from MNDNR no formal training in estimating scale age (Isermann et al. 2010c; McInerny et al. 2017). Interestingly, Ross et al. (2005) showed that experience does not always equate to competency in scale aging because age estimates by one inexperienced reader agreed better (94% compared to 76-87%) with known-age Black Crappie than ages estimated by two experienced readers. On the other hand, competency among whole otolith readers appeared unrelated to experience. Both inexperienced and experienced whole otolith readers almost always (99-100% of the time) correctly aged known age Black Crappies from Kentucky hatchery ponds (Ross et al. 2005), and Kruse et al. (1993) reported 97-98% age agreement among three inexperienced readers of whole otoliths from the two South Dakota populations.

Population-specific differences could also affect age precision and bias for both structures because growth rates and maximum ages differ among Black Crappie populations. For example, growth rates (based on scale ages and measurements) differed considerably among populations in Minnesota (McInerny and Cross 1999; 2008). Furthermore, Black Crappies in some Minnesota waters have also reached age 18 based on annuli counts on otolith cross-sections (MNDNR lake survey database), older than ages (ages 0-7) evaluated in previous studies. Ages estimated with annuli counts on either scales or whole otoliths could be less precise or more biased for populations composed of many individuals older than age 7. For other fish species, readers usually counted fewer annuli on scales or whole otoliths than on transverse views of otoliths when differences in age estimates occurred (Beamish 1979; Hoyer et al. 1985; Skurdal et al. 1985; Beckman 2002).

Timing of annulus appearance and sex of Black Crappie could also affect precision and bias of scale and whole otolith age estimates. Most age structures from Black Crappie are collected during routine surveys in June, July, or August (MNDNR 1993; 2017), and the newest annuli usually appear at some unknown time during summer. Thus, inconsistent interpretation of the structure edge will reduce precision of age estimates. Timing of annulus appearance could also differ between cellular scales and acellular otoliths because mechanisms creating annuli and other marks on these structures differ (Popper and Zu 2000; Isely and Grabowski 2007). Furthermore, scale and otolith annuli from younger centrarchids appeared earlier in the growing season than from older centrarchids (Beckman 1940; Crawford et al. 1989). Although both sexes mature at similar lengths (~ 150 mm TL) and ages (2 to 3) and exhibit similar mortality, males build and defend nests and often grow slightly faster than females in Minnesota (Isermann et al. 2010a; 2010b; McInerny 2014). These life history differences could cause different spacing patterns between annuli, cause checks, or affect timing of annuli appearance on structures more so in one sex than the other.

Because they were linked with growth, precision and bias could also differ regionally or differ among lakes with differing productivity, depth, and size within Minnesota. Black Crappie populations occur in roughly 2,400 water bodies throughout the state with variable growing seasons and lake productivity. Mean yearly air temperatures range from 1.7 °C in the northeast to 8.3 °C in the south, and total phosphorus concentrations vary from < 10 µg/L to nearly 500 µg/L (Heiskary and Wilson 1989; Seeley 2006). McInerny and Cross (1999; 2008) found that back-calculated lengths at scale ages 1, 2, 3, 4 or 5 increased with increasing total phosphorus concentrations, and declined with increasing latitude, decreasing longitude, increasing lake depth, and decreasing lake surface areas in Minnesota. In southern Minnesota, density-dependent growth could also have been occurring because growth in lakes with gill net catch per lift (CPUE) of Black Crappie > 3.8 was slower than growth in lakes with lower gill net CPUE (McInerny and Cross 1999).

Our study objectives included determining between-reader precision and age-bias of ages of Black Crappie estimated with scales and whole otoliths. We then tested for effects of age (from transverse views of otoliths), population, timing of sample collection, sex, latitude, longitude, lake depth, lake surface area, and gill net CPUE on precision and bias. Our last objective was to define the age ranges that can be reliably estimated with scales and whole otoliths.

METHODS

Data collection

Scales and sagittal otoliths of Black Crappie were collected from at least 10 individuals per population during summer and fall 1989-1991 and from April through October 2001 through 2003. Most Black Crappie for this study were caught with standard gill netting or trap netting during June, July, or August (MNDNR 1993; 2017), but some were caught with trap netting in September and October, winter angling, or boom electrofishing in May. We measured to the nearest mm total length, scales were removed from the left side just posterior of the depressed pectoral fin, and both sagittal otoliths were collected. Scales and otoliths were placed in individually labeled coin envelopes and air-dried. We determined sex by direct examination of gonads for all Black Crappie captured with angling and all those sampled in 2002 and 2003.

Each structure required different processing before estimating age. Impressions of scales on cellulose acetate were made with the aid of a heated hydraulic shop press. Microfiche readers were then used to magnify scale images in order to reveal annuli. Whole otoliths were first placed in a clear glass dish filled with water or ethanol (one reader used water and the other used ethanol). This dish was then placed on a black stage plate underneath a stereo microscope, and then illuminated with reflected white light before counting annuli. We estimated age by counting annuli on each structure; cutting and spacing patterns on scales, and opaque, narrow bands on whole views of otoliths (Long and Grabowski 2017; McInerny 2017). Readers knew the date of sample collections but did not have access to any other information that could aid their estimates of age.

We attempted to minimize as best as practicable error caused by differences in reader abilities by recruiting experienced, competent readers. Thus, all readers in this study received in the past either formal or informal training from other competent readers of the appropriate structure. Readers also had at least 10 years of practical experience that included comparisons of age estimates made with other structures, comparisons with modes of length-frequency distributions, and comparisons of ages from the same structure estimated by other competent readers. Because participants worked at different locales, readers used different microfiche readers and microscopes.

To estimate age-bias, one reader estimated age by counting annuli on transverse views of otoliths exposed by the crack-burn method (CB otoliths) months after completing age estimates from scales and whole otoliths (Barber and McFarlane 1987). The crack-burn method involved placing the otolith in the palm of one hand, snapping it in half by applying thumbnail pressure at the kernel area, and then placing the broken edge over a candle flame until turning brown. The unburned edge was then inserted into clay followed by applying a drop of mineral oil on the burned edge. After illuminating with white light under a stereo microscope, the reader, experienced with this method, estimated age of each crappie by counting narrow bands (Long and Grabowski 2017). Annuli counts on transverse views of Black Crappie otoliths have been assumed to provide the most accurate age estimates when known-age Black Crappie are not available (Schramm and Doerzbacker 1985; Crumpton et al. 1988; Isermann et al. 2010c).

Data analyses

We used a combination of between-reader agreement, mean coefficients of variation (CV) of age between readers, and agreement and age-bias as a function of CB otolith age to evaluate scales and whole otoliths as age structures for Black Crappie. We calculated between- reader agreement (%) for the entire sample, by CB otolith age, and by population (Chang 1982; Campana et al. 1995). We used 80% agreement as a benchmark because this value represents the minimum acceptable agreement for many age structure evaluations (Maceina et al. 2007). We then calculated between-reader mean CV of age for the entire sample and by population. We determined mean CV for each age structure by first calculating CV of the two ages estimated for each individual Black Crappie, summing all CVs, and then dividing by the total number of Black Crappie aged in the particular group (Chang 1982). For each structure, we tested for between-reader age-bias by calculating mean age ± 95% confidence limits estimated by one reader as a function of the age estimated by the other and vice versa (Campana et al. 1995). We concluded reader age-bias occurred if one reader consistently counted more annuli (95% confidence intervals do not overlap) than the other. To determine agreement and age-bias as a function of CB otolith age, we compared age estimates with each structure to those ages estimated with CB otoliths. We calculated for each reader of each structure age-bias (years) by subtracting CB otolith age from each age estimate.

We used linear mixed-effects models to test for the effects of population and CB otolith age on between-reader agreement, agreement between scale or whole otolith age and CB otolith age, and age-bias as a function of CB otolith age. We set as a random independent variable *population* to account for differences in sample size along with agreement and bias rates among Black Crappie populations. Crack-burn otolith age was set as a fixed independent variable; we excluded from analyses those CB otolith ages represented by a single individual from the entire sample or if a unique CB age was found in only one population. We tested for effects of sex on a reduced data set that lacked all unsexed crappies. Bias-corrected Akaike Information Criteria (AICc) coupled with the examination of *t*-or *z*-statistics were used to select the best fitting model. We report only the model with the lowest AICc (Burnham and Anderson 2002); in cases where two models had AICc differences < 2, we chose the simpler model. We also concluded that independent variables with *t*- or *z*- statistics > 2 or < -2 have strong positive or negative influences on the dependent variable (Luke 2017). We used the *lme4* package in *R* (version 3.6.2) for all modeling (Bates et al. 2015; R Core Team 2019).

Because all except two populations were sampled only once, we used a two-step process to test for effects of maximum depth, lake surface area, latitude, longitude, gill net CPUE of Black Crappie, and sample month on between-reader agreement, agreement with CB otolith age, and age-bias as a function of CB otolith age. First, the *ranef* function in *lme4* was used to extract for each population the best linear unbiased predictors (BLUPs) of realized random effects if the best (lowest AICc) mixed-effects model suggested that the dependent variable was affected by population (Bates et al. 2015). Then, separate for independent variables maximum depth, lake surface area, latitude, longitude, and gill net CPUE of Black Crappie, we used linear regressions to determine if population BLUPs were associated with the particular independent variable. Lake surface area was log-transformed to improve normality. Universal Transverse Mercator (UTM; Zone 15) northing described latitude, and UTM easting described longitude. Sample month was partitioned into June, July, August, and September-May, and effects of this variable on population BLUPs were analyzed with one-way analysis of variance (ANOVA). We felt confident that the structure edge in the September-May period would be interpreted correctly (the structure edge was not an annulus on scales and otoliths collected in September or October, the structure edge was an annulus on structures collected from January to May).

RESULTS

MNDNR staff collected scales and sagittal otoliths from 933 Black Crappies from 49 populations, and these populations were from a wide variety of lakes spread across Minnesota. Sample sizes ranged from 10 to 46 per population; median sample size per population equaled 18. Median total length of Black Crappie was 202 mm, and lengths ranged from 78 to 334 mm. Sex was determined for 559 (60% of total) Black Crappie from 30 populations, 295 of which were female. Two hundred forty one Black Crappies were collected in June, 274 in July, 205 in August, and 213 in September-May. The median lake surface area equaled 277 ha and ranged from 21 to 5,750 ha, and maximum depths ranged from 1.2 to 34 m; median = 9.4 m. The maximum longitudinal distance between lakes was 306 km, and the maximum latitudinal distance was 481 km. Crack-burn otolith age ranged from zero to 17, but only five CB otolith age 0 (all from the same population), one CB otolith age 11, and one CB otolith age 17 were observed. The CB otolith age range of the remaining Black Crappie was 1 to 9.

Between-reader precision

Lower overall precision was found between scale readers than between whole otolith readers. Scale age estimates ranged from zero to 11, and whole otolith age estimates ranged from zero to 16. For all samples combined, age agreement between scale readers averaged 86% compared to 90% age agreement between whole otolith readers. Similarly, mean CV between scale readers was 2.9% compared to 2.2% between whole otolith readers. No consistent age-bias occurred between scale readers, but Reader 1 counted fewer annuli at whole otolith ages 8 and 9 than Reader 2 (Figure 1). Because this inconsistency resulted from annuli counts from one slow growing population (mean CB otolith age = 8.9; range 6 to 9; mean total length = 160 mm) that composed 56% of the CB otolith ages 8 and 9 observed in this study, we concluded no overall age-bias occurred between whole otolith readers.

Between-reader agreement declined with increasing age and differed among the 49 populations. Agreement between scale readers equaled or exceeded 80% up to age 3, but gradually declined with increasing CB otolith ages 4 and older, and agreement between whole otolith readers equaled or exceeded 80% up to age 7 (Figure 2). Agreement between scale readers ranged from 40 to 100% among populations (median agreement = 91%), whereas agreement between whole otolith readers ranged from 33 to 100% among populations (median = 96%) (Figure 2). Scale age agreement equaled or exceeded 80% in 78% of the populations while whole otolith agreement equaled or exceeded 80% in 88% of the populations (Figure 2). Lastly, mean CV of age between scale readers ranged from zero to 20.2% among populations (median = 1.2%) compared to zero to 25.6% among populations (median mean CV = 0.5%) between readers of whole otoliths.

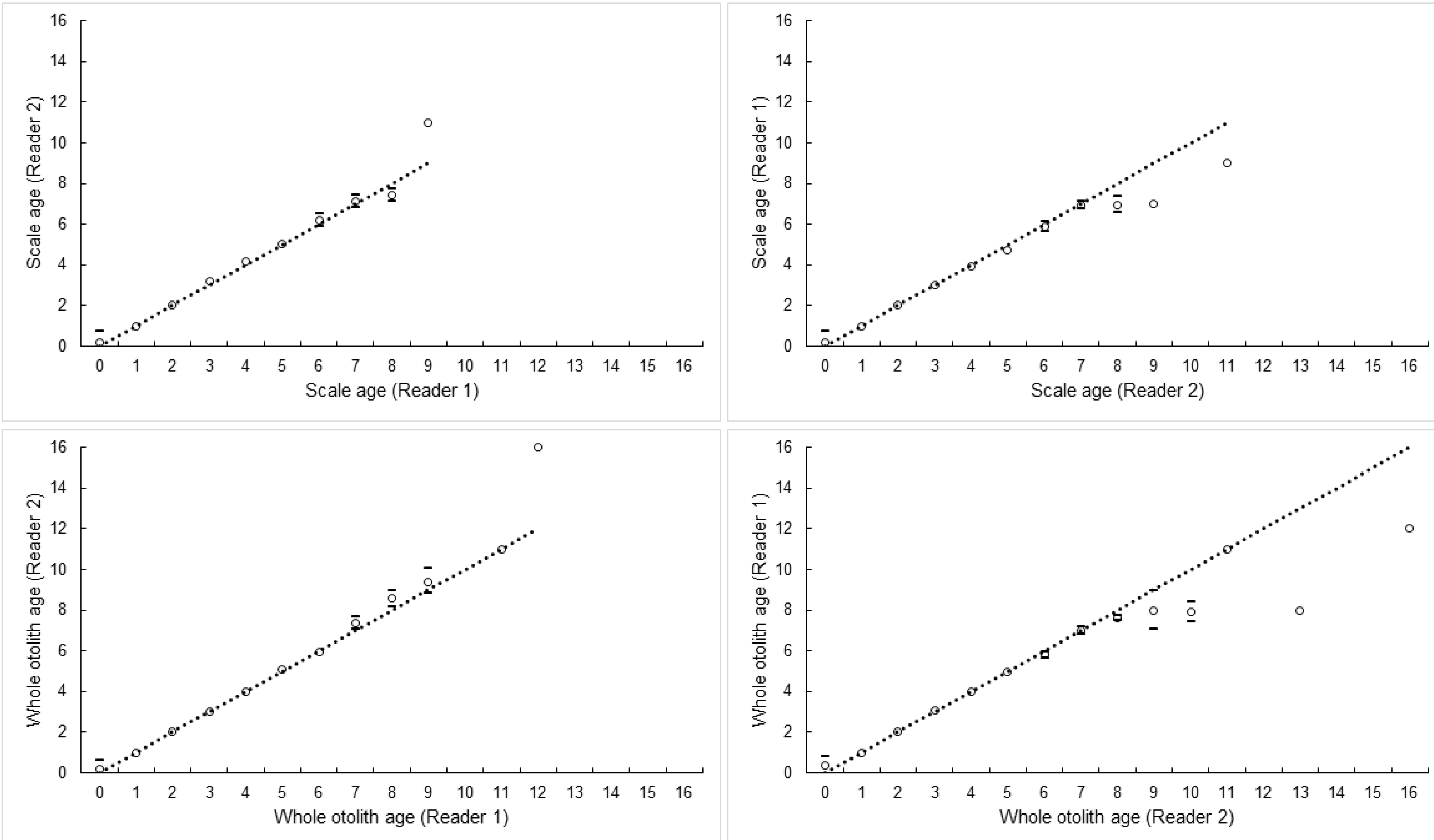

FIGURE 1. Mean age estimated by one scale reader as a function of the other and vice versa, and mean whole otolith age estimated by one reader as a function of the other and vice versa for 933 Black Crappies from 49 Minnesota populations (dotted line is line of equality; when shown, horizontal bars above and below open circles represent 95% confidence limits otherwise diameter of open circles included 95% confidence limits).

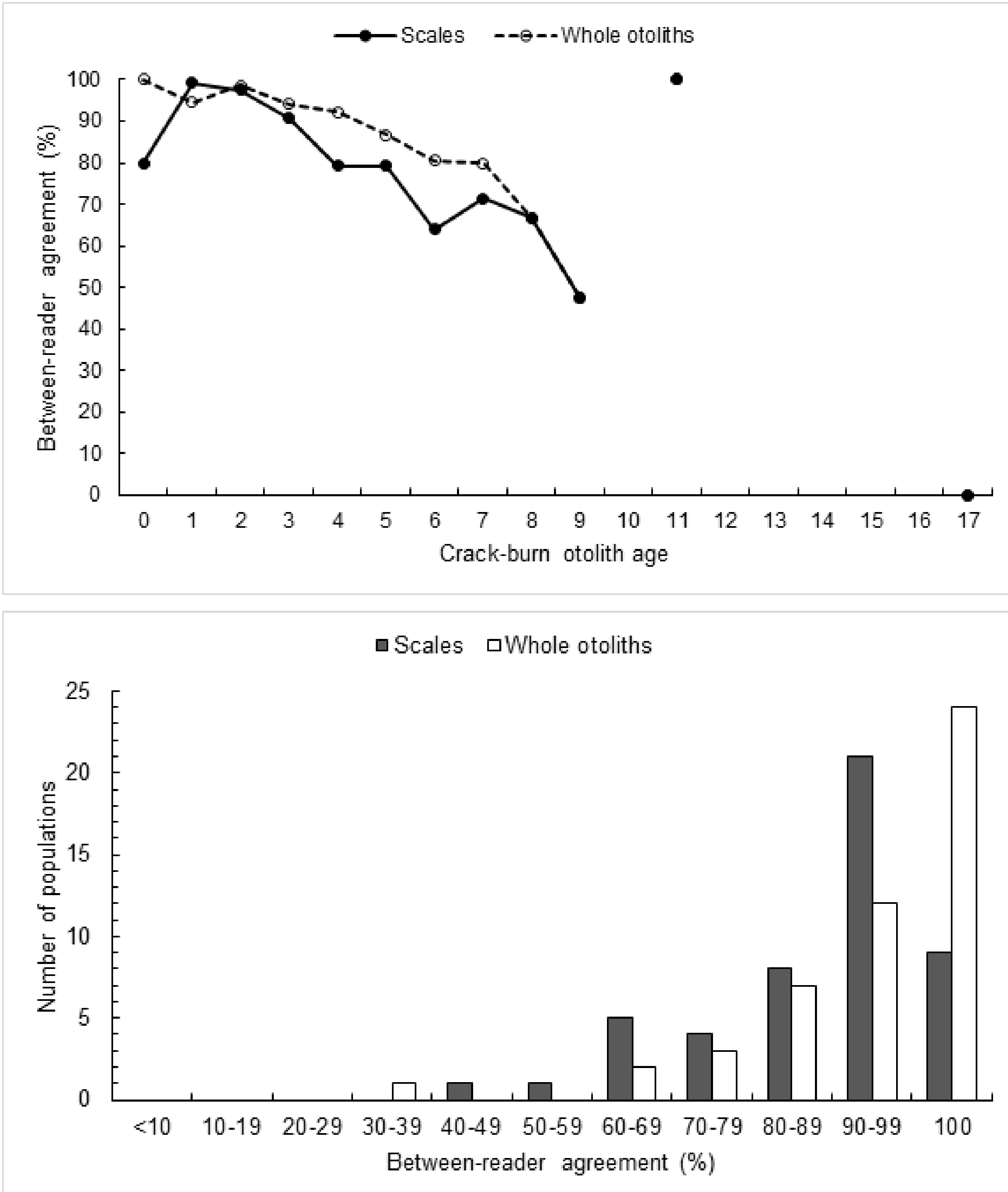

FIGURE 2. Between-reader agreement (%) of scale age estimates and between-reader agreement of whole otolith age as a function of crack-burn otolith age for 933 Black Crappies and distributions of percent between-reader agreement for each age structure among 49 Minnesota populations.

Logistic mixed-effects models strongly suggested that agreement between scale readers and agreement between whole otolith readers depended on both population and CB otolith ages 1 through 9 (Table 1). Modeling excluded CB otolith ages 0, 11, and 17 because these age groups were sampled from single populations. Linear regressions or ANOVAs of population BLUPs from the mixed-effect models suggested that agreement between scale readers and between whole otolith readers were not associated with gill net CPUE of Black Crappie, lake depth, lake surface area, UTM easting, UTM northing, or sample month (Table 2).

Sex also did not affect agreement between scale readers or whole otolith readers. Among-population medians and ranges of between-reader agreement of scale age appeared similar for females and males, and the same was true for between-reader agreement of whole otolith age (Table 3). Similarly, among-population medians and ranges of mean CV of age between scale readers and between whole otolith readers also appeared similar between sexes (Table 3). The logistic-mixed effects modeling of this reduced data set suggested that sex had negligible effects on age agreement between scale readers (z = - 0.570; n = 30; P = 0.5697; ΔAICc = + 1.8) and between whole otolith readers (z = - 0.079; n = 30; P = 0.9368; ΔAICc = + 2.0) compared to the interaction of population and CB otolith age.

TABLE 1. Akaike Information Criteria (AICc) scores and differences (ΔAICc) between the 'best' model (model with lowest AICc score) and other models for mixed-effects models testing effects of population and crack-burn (CB) otolith age (ages 1 through 9) on age agreement between readers of scales and whole otoliths, agreement between scale or whole otolith age and CB otolith age, and bias of scale age and whole otolith age as a function of CB otolith age for 926 Black Crappies from 49 Minnesota populations.

	Scales		**Whole otoliths**	
Model	AICc	ΔAICc	AICc	ΔAICc
Between-reader age agreement				
Population + CB otolith age	596.9	0	454.4	0
CB otolith age	639.3	42.4	507.8	53.4
Population	686.2	89.3	490.0	35.6
Age agreement with CB otolith age				
Population + CB otolith age	969.2	0	716.3	0
CB otolith age	1116.8	147.6	773.7	57.4
Population	1349.7	380.5	857.6	141.3
Age-bias as a function of CB otolith age				
Population + CB otolith age	2133.4	0	1055.3	0
CB otolith age	2410.8	277.4	1066.4	11.1
Population	2856.3	722.9	1213.9	158.6

TABLE 2. *t*- or *F*-statistics, *p*-values (*P*), and degrees of freedom (d.f.) for independent variable parameter estimates from linear regressions or one-way ANOVA testing the effects of gill net catch per lift (CPUE) of Black Crappie, maximum depth, surface area, Universal Transverse Mercator (UTM) easting and northing of 49 populations, and sample month on best linear unbiased predictors of population from the best mixed-effects models (Table 2) of age agreement between readers of scales and whole otoliths, agreement between scale or whole otolith age and CB otolith age (CB ages 1 through 9), and bias of scale age and whole otolith age as a function of CB otolith age for 926 Black Crappies from 49 Minnesota populations. *P* values < 0.05 are in bold.

	Scales			**Whole otoliths**	
Variable	Statistic	*P*	d.f.	Statistic	*P*
		Between-reader age agreement			
Gill net CPUE	*t* = -0.278	0.7821	1,47	*t* = -0.126	0.9001
Maximum depth	*t* = -0.872	0.3877	1,47	*t* = -1.427	0.1602
Lake surface area	*t* = -1.423	0.1614	1,47	*t* = -0.854	0.3974
UTM easting	*t* = 0.901	0.3720	1,47	*t* = 0.418	0.6779
UTM northing	*t* = 1.113	0.2712	1,47	*t* = -0.341	0.7345
Sample month	*F* = 0.905	0.4463	3,45	*F* = 0.899	0.4491
		Agreement with CB otolith age			
Gill net CPUE	*t* = -0.131	0.8966	1,47	*t* = -0.530	0.5985
Maximum depth	*t* = 0.271	0.7873	1,47	*t* = -1.365	0.1789
Lake surface area	*t* = -0.548	0.5864	1,47	*t* = -0.561	0.5777
UTM easting	*t* = 1.445	0.1550	1,47	*t* = -0.127	0.8995
UTM northing	*t* = 3.194	**0.0025**	1,47	*t* = 0.392	0.6972
Sample month	*F* = 0.233	0.8732	3,45	*F* = 0.754	0.5258
		Age-bias as a function of CB otolith age			
Gill net CPUE	*t* = 0.145	0.8857	1,47	*t* = 1.480	0.1456
Maximum depth	*t* = 0.145	0.8849	1,47	*t* = 0.145	0.8857
Lake surface area	*t* = -0.154	0.8786	1,47	*t* = -0.680	0.5000
UTM easting	*t* = 0.661	0.5117	1,47	*t* = -1.660	0.1036
UTM northing	*t* = 3.153	**0.0028**	1,47	*t* = 0.721	0.4747
Sample month	*F* = 0.233	0.2564	3,45	*F* = 0.019	0.9965

TABLE 3. Median (range in parentheses) between-reader agreement (%), between-reader mean coefficients of variation (CV; %), agreement (%) between scale or whole otolith age and crack-burn (CB) otolith age, and mean age-bias (difference in years between scale or whole otolith age and CB otolith age) by sex among 30 Black Crappie populations (total sample size = 559) in Minnesota.

	Scales		**Whole otoliths**	
	Females	Males	Females	Males
Between-reader agreement	91.7 (42.9 to 100)	96.4 (35.3 to 100)	100 (7.7 to 100)	100 (33.3 to 100)
Between-reader mean CV	1.3 (0 to 16.8)	0.4 (0 to 16.8)	0 (0 to 17.1)	0 (0 to 13.2)
Agreement with CB otolith age	85.2 (0 to 100)	86.6 (0 to 100)	92.8 (11.1 to 100)	100 (23.5 to 100)
Mean age-bias	-0.05 (-1.27 to 0.19)	0 (-1.94 to 0.45)	0 (-0.67 to 0.11)	0 (-0.81 to 0.01)

Comparisons with crack-burn otolith age

Whole otolith age estimates had better agreement with CB otolith age than scale age estimates, and percent agreement for both structures declined with increasing age and differed among populations. For all samples combined, age estimates from scales and CB otoliths agreed 78% of the time compared to 87% agreement between age estimates of whole otoliths and CB otoliths. Agreement between scale age and CB otolith age equaled or exceeded 80% at CB otolith ages 0 through 3, and then agreement dropped sharply with increasing CB otolith ages 5 and older (Figure 3). Conversely, agreement between whole otolith age and CB otolith age exceeded 80% for CB otolith ages 0 through 5, but agreement rapidly declined at CB otolith ages 8 and older (Figure 3). Agreement between scale age and CB otolith age ranged from zero to 100% among populations, and agreement equaled or exceeded 80% in 69% of the populations (Figure 3). On the other hand, agreement between whole otolith age and CB otolith age ranged from 23 to 100%, and agreement equaled or exceeded 80% in 82% of the populations (Figure 3).

Logistic mixed-effects modeling suggested that agreement of scale and whole otolith age estimates with CB otolith age were most affected by the interaction of CB otolith age (ages 1 through 9) and population rather than by each variable by themselves (Table 1). For both age structures, AICc of the model that included both independent variables was at least 57 AICc less than either model relating agreement with only population or only CB otolith age (Table 1). A linear regression suggested that population BLUPs of scale and CB otolith age agreement improved with increasing UTM northing, but BLUPs were not associated with gill net CPUE of Black Crappie, lake depth, lake surface area, UTM easting, or sample month (Table 2). For models between whole otolith and CB otolith age, population BLUPs were unassociated with these six independent variables (Table 2).

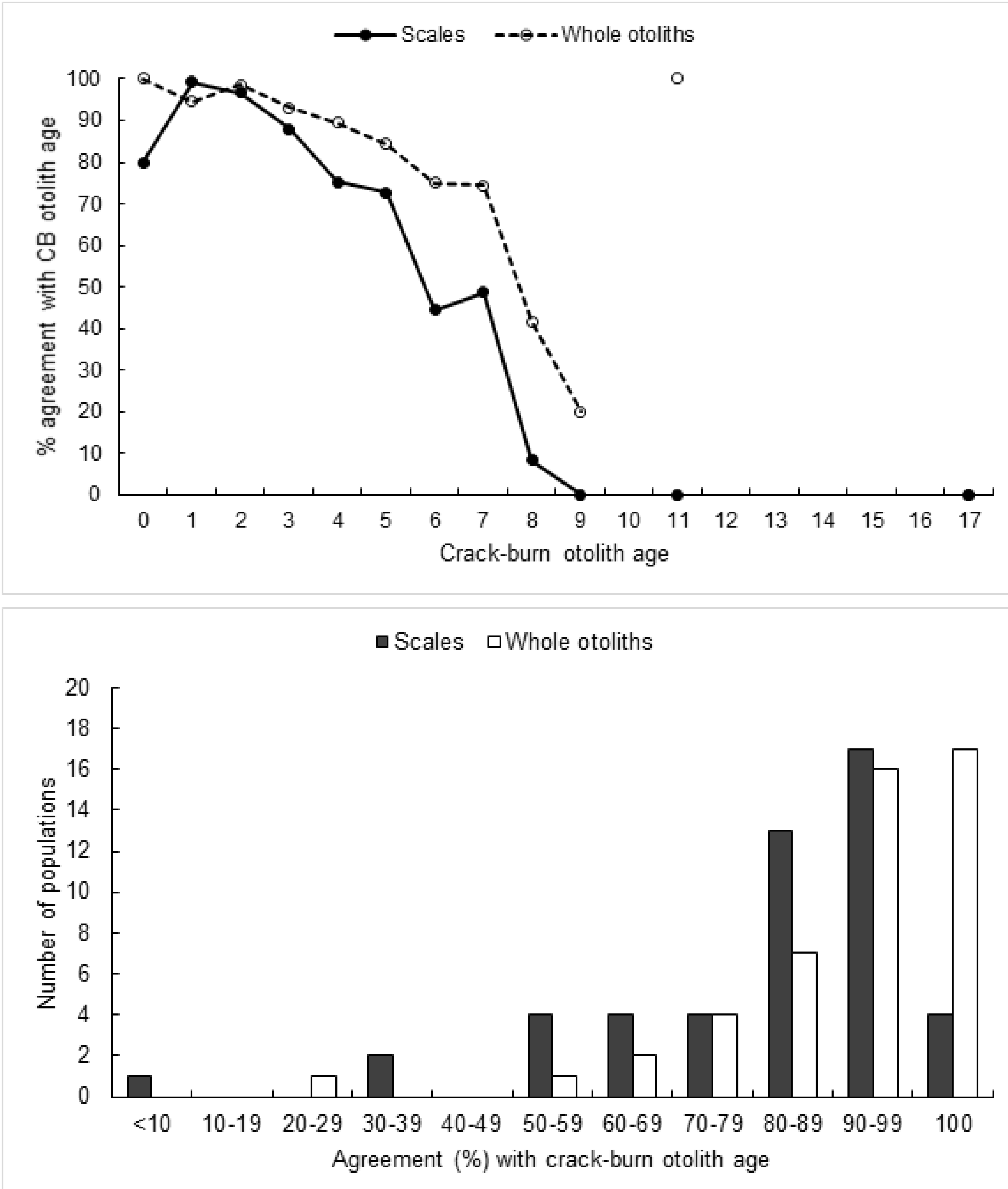

FIGURE 3. Agreement (%) between scale age estimates (both readers combined) and crack-burn (CB) otolith age and between whole otolith age estimates (both readers combined) and CB otolith age by CB otolith age of 933 Black Crappies and distributions of age agreement (%) among 49 Minnesota populations.

Although age estimates from both structures were relatively unbiased, whole otolith age estimates were less negatively biased with respect to CB otolith age than scale age estimates. Age-bias from both structures also increased with increasing CB otolith age and differed among populations. Scale age estimates were unbiased with respect to CB otolith ages 0 through 5, but showed increasing negative bias with increasing CB otolith ages 6 and older (Figure 3). Whole otolith age estimates appeared unbiased from CB otolith ages 0 through 7, but became negatively biased with respect to CB otolith ages 8 and 9 (Figure 4). Distributions of scale age-bias among populations showed four clearly negatively biased outliers; however, no clear outliers were observed in the distribution of whole otolith bias among populations (Figure 4).

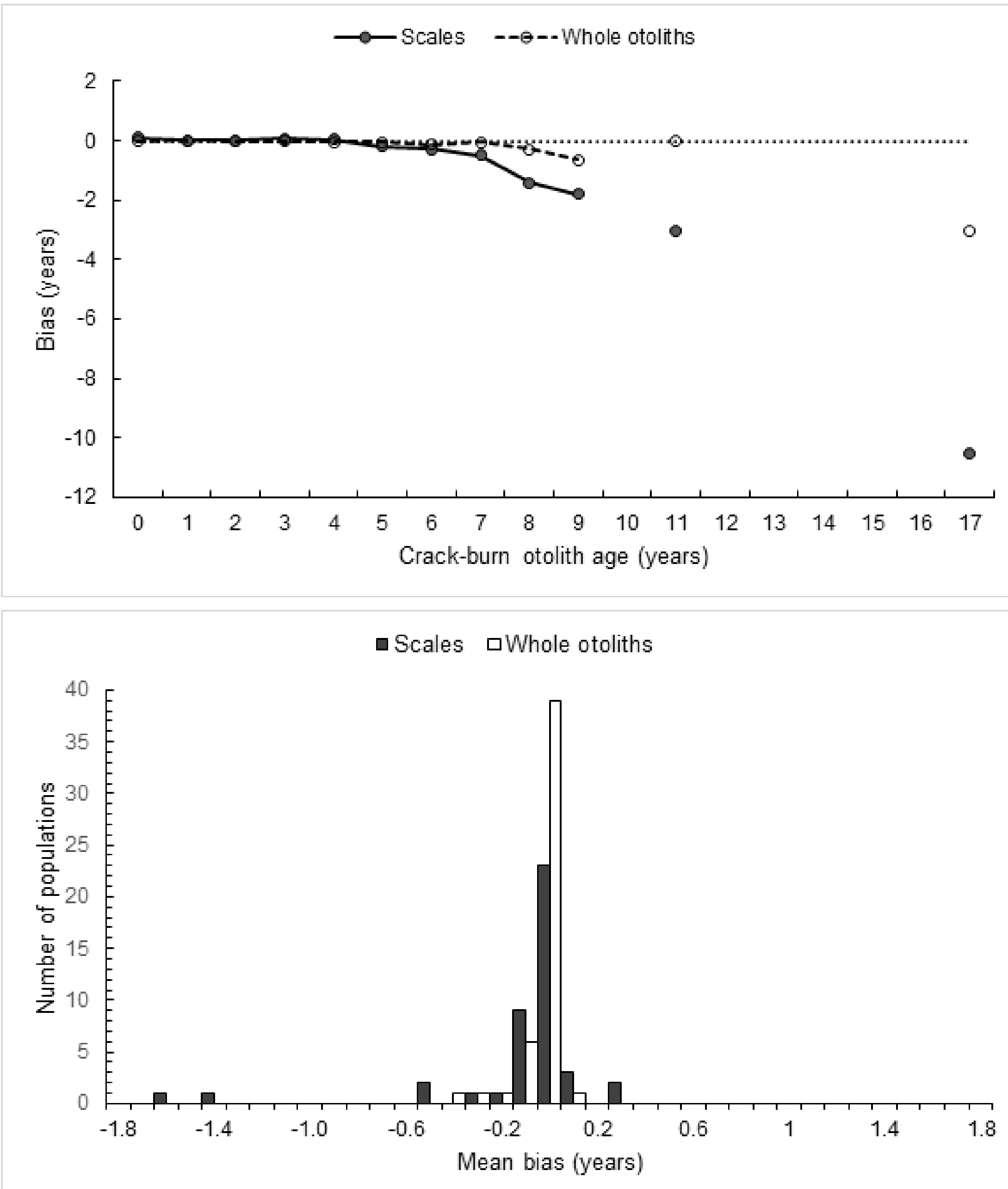

FIGURE 4. Mean age-bias (difference in years between scale or whole otolith age and crack-burn (CB) otolith age) of scale age estimates as a function of CB otolith age, mean bias of whole otolith age estimates as a function of CB otolith age for 933 Black Crappie, and distributions of mean scale age and whole otolith age-bias among 49 Minnesota populations.

Linear mixed-effects models indicated that scale and whole otolith age-bias as a function of CB otolith age was also most affected by the interaction of CB otolith age (ages 1 through 9) and population (Table 2). For both structures, AICc of the best models were 11.1 to 722.9 lower than models based on CB otolith age or population by themselves (Table 1). Linear analysis of population BLUPs from the best mixed-effect model suggested that scale age estimates from southern populations were more negatively biased than scale age estimates of northern populations, but scale age-bias was unassociated with the other five variables (Table 2). Analysis of population BLUPs suggested that age-bias of whole otolith estimates was unassociated with these six independent variables (Table 2).

Agreement with CB otolith age and age-bias as a function of CB otolith age was also unaffected by sex of Black Crappie. Among-population medians and ranges of agreement and age-bias between scale age and CB otolith age were similar for males and females (Table 3). Logistic mixed-effects modeling suggested that sex had negligible effects on age agreement between scale age and CB otolith age (z = - 1.312; n = 30; P = 0.190; $_{\Delta}$AICc = + 0.3), and linear mixed-effects modeling suggested that sex had no effect on scale-age bias as a function of CB otolith age compared to the interaction of population and CB otolith age (ages 1 through 9). Agreement and age-bias between whole otolith age and CB otolith age also appeared similar between sexes (Table 3). Similar to scales, logistic mixed-effects modeling suggested that agreement between whole otolith age and CB otolith age was unaffected by sex (z = - 1. 037; n = 30; P = 0.2997; $_{\Delta}$AICc = + 1.0) compared to the interaction of population and CB otolith age. Linear mixed-effects modeling showed that scale age-bias (t = 0.635; $_{\Delta}$AICc = + 1.6) and whole otolith age-bias (t = 1.016; $_{\Delta}$AICc = + 1.0) as a function of CB otolith age was also not affected by sex.

DISCUSSION

Our results suggested that annuli counts on whole otoliths were better than counts from scales for providing age estimates of Black Crappie, assuming annuli counts on otolith cross-sections were the most accurate method for estimating age of Black Crappie. However, because age-bias did not occur for either scales or whole otoliths until CB otolith age exceeded age 5, increasing sample sizes of scales and whole otoliths of Black Crappies younger than age 6 would offset aging error for estimating growth metrics such as length at age. Because declines in between-reader precision, declines in agreement with CB otolith age, and increased age-bias as a function of CB otolith age began at ages four or older, error in scale and whole otolith age was probably caused by increased crowding of annuli near structure edges. Lastly, although arguably the best method for estimating age of Black Crappie in this study (Schramm and Doerzbacker 1985; Crumpton et al. 1988; Isermann et al. 2010c), some unknown amount of error in CB otolith age probably occurred because of occasional poor breaks or insufficient burns.

The strong association between UTM northing on agreement between scale and CB otolith age and scale age-bias among populations was probably unrelated to distinct winters, but could be a function of latitudinal differences in water chemistries. Kruse et al. (1993) and Hoxmeier et al. (2001) hypothesized that distinct winters contributed to clearer annuli on Black Crappie and Bluegill *Lepomis macrochirus* scales; thus, explaining the better between-reader precision of scale ages in northern populations. However, Minnesota lakes were ice covered for at least three months each year; thus, all study populations experienced distinct winters. Additionally, the 59-100% agreement between scale age and whole otolith age or known-age Black Crappie in Kentucky, North/South Carolina and Florida (Schramm and Doerzbacker 1985; McInerny 1989; Ross et al. 2005) was similar to the range of agreements in this study, suggest that clarity of scale annuli was not affected by length of winters. Water chemistry parameters including conductivity, total alkalinity, turbidity, and concentrations of sulphate, chloride, total phosphorus, and total nitrogen in Minnesota lakes decreased with increasing latitude (Moyle 1956; Heiskary and Lindon 2010). Distributions of species of fish and aquatic macrophytes have been linked to different water chemistries among Minnesota lakes (Moyle 1956); thus, formation of Black Crappie scales could also be affected in some unknown way by different water chemistries.

Differences in microscope optics could have lowered precision between readers of whole otoliths because we later learned that the optics of the microscope used by Reader 1 were inferior to those of the microscope used by Reader 2. The poor optics in the one microscope probably decreased Reader 1's ability to distinguish crowded annuli from translucent backgrounds near the edge of otoliths from some older Black Crappies. Optical qualities of the two microfiche readers used for scale age estimates were similar; thus, use of different microfiche readers did not affect between-reader precision and age-bias of scale age estimates.

Effects on precision and age-bias from variable lake surface area, lake depth, UTM easting, sample month, and sex could have been masked by the other variables examined in this study. For example, the population with the poorest agreement between scale age and CB otolith age and between whole otolith age and CB otolith age inhabited the second smallest lake with the third highest gill net CPUE among the study lakes. Samples were also collected in July; thus, annulus appearance in this slow growing population (mean length = 160 mm; mean age = 8.9), may not have been completed by then. Thus, effects associated with lake size, gill net CPUE, or sample period could have affected precision and bias of scale and whole otolith age estimates of this population but not all populations. With the exception of sex, each variable was fixed to a particular population; thus, we could not control for the effects of one variable with respect to the others. Lastly, because of their experience and prior training, our scale and whole otolith readers usually recognized checks and other aberrations on scales and whole otoliths that could have led to erroneous age estimates.

MANAGEMENT IMPLICATIONS

Our study suggested that age of Black Crappie should be estimated with annuli counts on otoliths, and annuli counts on some transverse views of otoliths should be made to support estimates made with annuli counts on whole otoliths. While whole otoliths provided better age estimations than scales for most populations, scales performed well enough to remain a viable option for some populations if readers are properly trained. However, managers should still collect otoliths from some individuals so that their scale age estimates can be supported with annuli counts on otoliths. Black Crappie fisheries worth managing in Minnesota will also have sufficient abundance to support sampling for age estimations. Median annual angler harvest of Black Crappie in Minnesota was 810 (range = 0 to 1,000,000) annually per water body (MNDNR creel survey database); thus, the number of crappies needed to be sacrificed for age-growth analysis would usually be negligible with respect to angler harvest.

ACKNOWLEDGMENTS

T. K. Cross and J. R. Reed, Minnesota Department of Natural Resources, assisted with sample collection in fall 1989, 1990, and 1991, and staff from Minnesota Department of Natural Resources collected most of the Black Crappies during summer 1989 and summer, 2001 through 2003. D. Logsdon, S. Shroyer, and two anonymous reviewers critiqued earlier drafts, and their comments were incorporated into this report.

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