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ARTICLE

# Precision of Four Otolith Techniques for Estimating Age of White Perch from a Thermally Altered Reservoir

**Richard A. Snow and Michael J. Porta**
*Oklahoma Department of Wildlife Conservation, Fisheries Research Laboratory, 500 East Constellation, Norman, Oklahoma 73072, USA*

**James M. Long***
*U.S. Geological Survey, Oklahoma Cooperative Fish and Wildlife Research Unit, Department of Natural Resource Ecology and Management, 007 Agriculture Hall, Oklahoma State University, Stillwater, Oklahoma 74078, USA*

***Abstract***
**The White Perch *Morone americana* is an invasive species in many Midwestern states and is widely distributed in reservoir systems, yet little is known about the species' age structure and population dynamics. White Perch were first observed in Sooner Reservoir, a thermally altered cooling reservoir in Oklahoma, by the Oklahoma Department of Wildlife Conservation in 2006. It is unknown how thermally altered systems like Sooner Reservoir may affect the precision of White Perch age estimates. Previous studies have found that age structures from Largemouth Bass *Micropterus salmoides* and Bluegills *Lepomis macrochirus* from thermally altered reservoirs had false annuli, which increased error when estimating ages. Our objective was to quantify the precision of White Perch age estimates using four sagittal otolith preparation techniques (whole, broken, browned, and stained). Because Sooner Reservoir is thermally altered, we also wanted to identify the best month to collect a White Perch age sample based on aging precision. Ages of 569 White Perch (20–308 mm TL) were estimated using the four techniques. Age estimates from broken, stained, and browned otoliths ranged from 0 to 8 years; whole-view otolith age estimates ranged from 0 to 7 years. The lowest mean coefficient of variation (CV) was obtained using broken otoliths, whereas the highest CV was observed using browned otoliths. July was the most precise month (lowest mean CV) for estimating age of White Perch, whereas April was the least precise month (highest mean CV). These results underscore the importance of knowing the best method to prepare otoliths for achieving the most precise age estimates and the best time of year to obtain those samples, as these factors may affect other estimates of population dynamics.**

Temperature is an important environmental factor affecting fish growth, reproduction, physiological behavior, immune system function, mortality, and early life history (Beitinger et al. 2000; Dhillon and Fox 2004; Stewart and Allen 2014). Water temperature is often altered around impoundments. Hypolimnetic releases into downstream tailwaters often depress water temperature, slowing fish growth (Clarkson and Childs 2000) and constricting daily growth increments in otoliths (Bestgen and Bundy 1998; Song et al. 2009; Porta 2011). Conversely, hot water releases used to cool equipment in power plant reservoirs elevate the water temperature. This often increases fish growth (Perry and Tranquilli 1984), but false annuli can form in otoliths during high-temperature stress and can confound age estimates for resident species (Hales and Belk 1992; Albert et al. 2009).

In thermally altered systems, one would expect age estimation to be more variable because annual rings may be

*Corresponding author: longjm@okstate.edu
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faint due to a lengthened growing season or accessory checks may be counted as annuli. This phenomenon was observed when estimating ages of Largemouth Bass *Micropterus salmoides* by using scales, as annuli were irregular and inconsistent due to accessory check formation (Galloway and Kilambi 1988). Similarly, Perry and Tranquilli (1984) presumed that scales and whole otoliths were unreliable for age estimation because of the presence of false annuli, leading them to use sectioned otoliths for age estimation. Bluegills *Lepomis macrochirus* collected from a thermally altered reservoir exhibited check formation on otoliths but only in a small proportion of the age sample (Hales and Belk 1992). These factors that increase age estimate variability could lead to errors in assigning ages and subsequent misunderstanding of population dynamics.

The White Perch *Morone americana* is an invasive species in the Midwest, including Oklahoma, and is widely distributed in reservoir systems, yet little is known about the species' age structure and population dynamics. White Perch ages have commonly been estimated with scales (St. Pierre and Davis 1972; Schaeffer and Margraf 1986) but have only recently been estimated with otoliths (Kuklinski 2007; Feiner et al. 2012; Bethke et al. 2014; Porta and Snow 2017a). Estimating the ages of White Perch in Oklahoma by use of otoliths was considered dependent on preparation method (whole and broken), but these differences were not assessed statistically (Kuklinski 2007). Based on the use of sectioned otoliths, White Perch ages were validated with marginal increment analysis to form one annulus per year through age 8 in Sooner Reservoir, a thermally altered system in Oklahoma (Porta and Snow 2017a). Additionally, timing of annulus formation varied among age-groups, with younger fish forming annuli in April, whereas older fish formed annuli in June (Porta and Snow 2017a); such differences could influence interpretation of annuli in future research depending on when data are collected.

Counting annuli in White Perch otoliths from this thermally altered system could be made more precise with additional preparation techniques (e.g., burning and staining) that enhance contrast. Both burning and staining enhance the visibility of winter growth zones (Richter and McDermott 1990) and have been used for a variety of fish species (Bouain and Siau 1988; Richter and McDermott 1990; Secor et al. 1992; Peltonen et al. 2002; Mauck and Boxrucker 2004; Frenette and Snow 2016). Burning and staining appear to provide comparable results even though burning is quicker and less laborious than staining; however, attention to temperature is important because overheating can destroy an otolith (Richter and McDermott 1990).

Our objective was to quantify the precision of age estimates for White Perch in Sooner Reservoir by using sagittal otoliths and four otolith preparation methods: whole, broken, browned, and stained. In addition, because of the altered thermal regime and differences in timing of annulus formation among ages, we sought to identify whether precision estimates varied among months when samples were taken. This study will provide valuable insight on otolith preparation and the timing of fish collection to achieve the most precise age estimates possible.

## STUDY AREA

Sooner Reservoir is a 2,185-ha impoundment in Pawnee and Noble counties, north-central Oklahoma, with a maximum depth of 27 m and an average depth of 8.5 m (Figure 1). The reservoir was built in 1972 to serve as a cooling reservoir for a coal-fired power plant owned by Oklahoma Gas and Electric Company. Water for cooling is managed by inflow and discharge canals, with water coming from the Arkansas River at the dam and discharging at the opposite side. Unpublished water quality monitoring data from the Oklahoma Water Resources Board (OWRB) at stations 1–3 indicate the degree of water temperature alteration as a result of cooling activities. In general, water temperature is elevated at the end of the discharge canal (OWRB station 3), is moderated at midlake (OWRB station 2), and returns to nominal temperature at the dam (OWRB station 1), where river water is drawn into the reservoir (Figure 2). Depending on the month, mean water temperature can be up to 5°C higher at the discharge than the inflow of the dam. At peak capacity, the heated effluent can be up to 11°C above ambient (Gilliland 1981).

White Perch were first observed in Sooner Reservoir by the Oklahoma Department of Wildlife Conservation (ODWC) during standardized sampling in 2006 (Copeland 2016); they likely came from the nearby Arkansas River as part of water withdrawals for plant operation. White Perch have become the most abundant fish species in the reservoir since their discovery.

## METHODS

White Perch were sampled monthly by using boat electrofishing (pulsed DC, high voltage; Smith-Root 7.5 GPP) and experimental gill nets from June 2015 through April 2016 at randomly selected sites throughout the reservoir (Porta and Snow 2017a). The experimental gill nets were 24.4 m long × 1.8 m deep and were composed of eight 3-m panels (12.7-, 15.875-, 19.05-, 25.4-, 38.1-, 50.8-, 63.5-, and 76.2-mm bar mesh). After sampling each month, a subset ($N \geq 25$) of fish was randomly chosen from all sampling areas for age estimation; those fish were measured for TL, and their sagittal otoliths were removed for later examination in the laboratory.

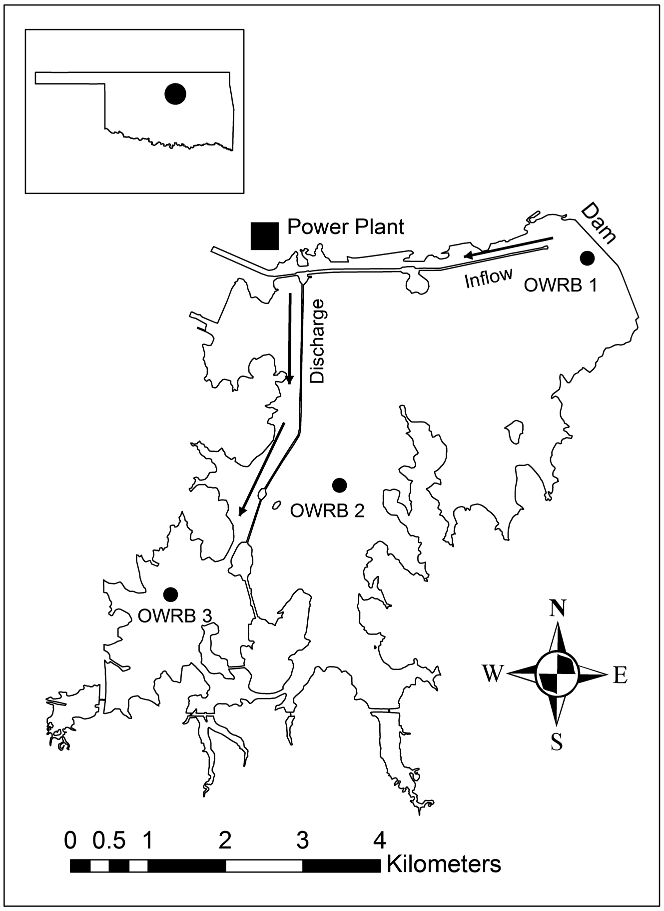


FIGURE 1. Map of Sooner Reservoir, a cooling reservoir for a coal-fired power plant built in north-central Oklahoma in 1972. Water is drawn into the plant from the dam and is discharged along the west side as indicated by arrows. Stations 1–3 for water temperature data taken by the Oklahoma Water Resources Board (OWRB) periodically between August 1995 and April 2015 are indicated (see Figure 2).

Ages were estimated using four otolith preparation methods in the following order: whole, broken, browned, and stained. Estimates were completed (independent estimates, concert agreements) for a given method before attempting estimation for another method. To avoid systematic bias, otoliths were randomized prior to conducting each method. Age was estimated from one whole otolith of each pair before being broken in half perpendicular to the longest axis (Buckmeier and Howells 2003). Next, both sides were ground flat by using a rotary tool fixed with a grinding bit (Number 85422; Dremel, Racine, Wisconsin). The rotary tool was attached to a table in a vise, and plastic-coated forceps were used to securely hold the otolith during the grinding process. The broken portions of the otoliths were polished using wet, 1,600-grit sand paper (Maceina 1988; Buckmeier and Howells 2003), and age was estimated from both sides. If the age estimates differed between sides, the otolith was discarded from

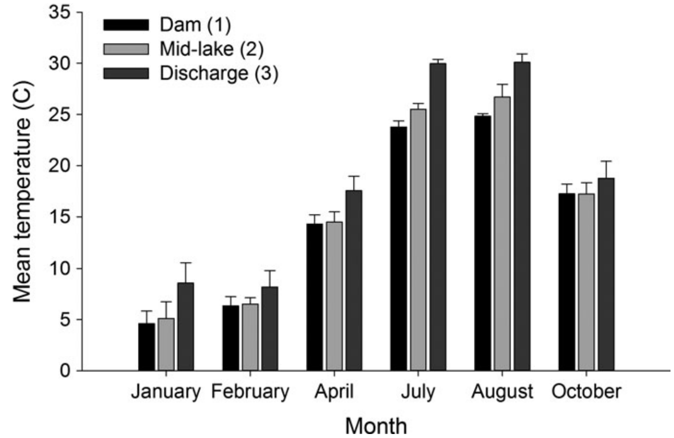


FIGURE 2. Mean (+SE) water temperature of Sooner Reservoir at three long-term monitoring stations (data courtesy of Oklahoma Water Resources Board) from August 1995 to April 2015. See Figure 1 for locations of monitoring sites.

further analysis. Afterward, one side was browned at 104°C using a hot plate, and the other side was stained using a solution of neutral red (1%) and acetic acid (5%) in distilled water (Richter and McDermott 1990; Peltonen et al. 2002). The browning technique increases the contrast between the accretion and discontinuous zones (Secor et al. 1992; Mauck and Boxrucker 2004), whereas the dyeing technique stains the protein that aggregates within the annual ring, which is viewed as a narrow, dark-red band (Peltonen et al. 2002). If age could not be estimated from any of the four techniques from one of the otolith pairs, then the other otolith was used.

Two readers independently examined the otoliths to estimate ages (Hoff et al. 1997) by using a variable-power dissection microscope (4–45×). Otoliths were selected at random without reference to fish length. Otoliths were placed in clay, submerged in water, and illuminated with a fiber optic filament. For each reader, annuli were first counted from the nucleus to the outer edge; this procedure was repeated to verify the first count, and age was then estimated. Agreements in age between readers were considered the age of the fish. If readers disagreed on fish age, both readers viewed the otoliths on a high-resolution monitor connected to an optic-mount digital camera attached to the microscope, and an age was agreed upon.

Precision of age estimates was evaluated between the readers' independent estimates and for each technique using agreed-upon age estimates by calculating the coefficient of variation (CV; Chang 1982; Kimura and Lyons 1991). Reader bias plots (mean age assigned by one reader for each age assigned by another reader; Campana et al. 1995) were generated. The CV of age estimates (rank value) was tested for differences among techniques and among sampling months (main effects) with a two-factor ANOVA (Kruskal–Wallis equivalent) using SAS software (GLM procedure) followed by Tukey's honestly significant difference test to determine where differences occurred. Precision estimates (i.e., CV) were plotted against estimated age for each otolith preparation technique to look for potential trends.

To examine the effect of precision on resulting growth models for the entire population, von Bertalanffy growth model parameters were estimated using age data from each of the methods and the month that provided the highest precision. Because these coefficients are highly intercorrelated (Ogle 2016), rather than calculate 95% confidence intervals for parameter estimates, we calculated the SE of the regression (SER) for each model as a measure of predictive performance:

$$\mathrm{SER} = \sqrt{\sum (Y - Y')^2 / N},$$

where $Y$ and $Y'$ are the observed and predicted TLs for an individual and $N$ is the number of individuals used in the model. We also used the resulting equations to predict TL at age (through the maximum age estimated for the technique) and calculated (1) the maximum difference in TL at age and (2) the CV among techniques for each age as a measure of differences in growth models. This approach provided more intuitive and interpretable results than equations that might present statistically different results that were not biologically meaningful. All statistical analyses were considered significant at $P \leq 0.05$.

## RESULTS

We estimated ages for 573 White Perch ranging from 20 to 308 TL mm. Ages could not be agreed upon for 5 whole otoliths, 10 browned otoliths, and 10 stained otoliths. Ages from broken, stained, and browned otoliths ranged from 0 to 8 years; however, whole-view otolith ages ranged from 0 to 7 years. Broken otoliths were the

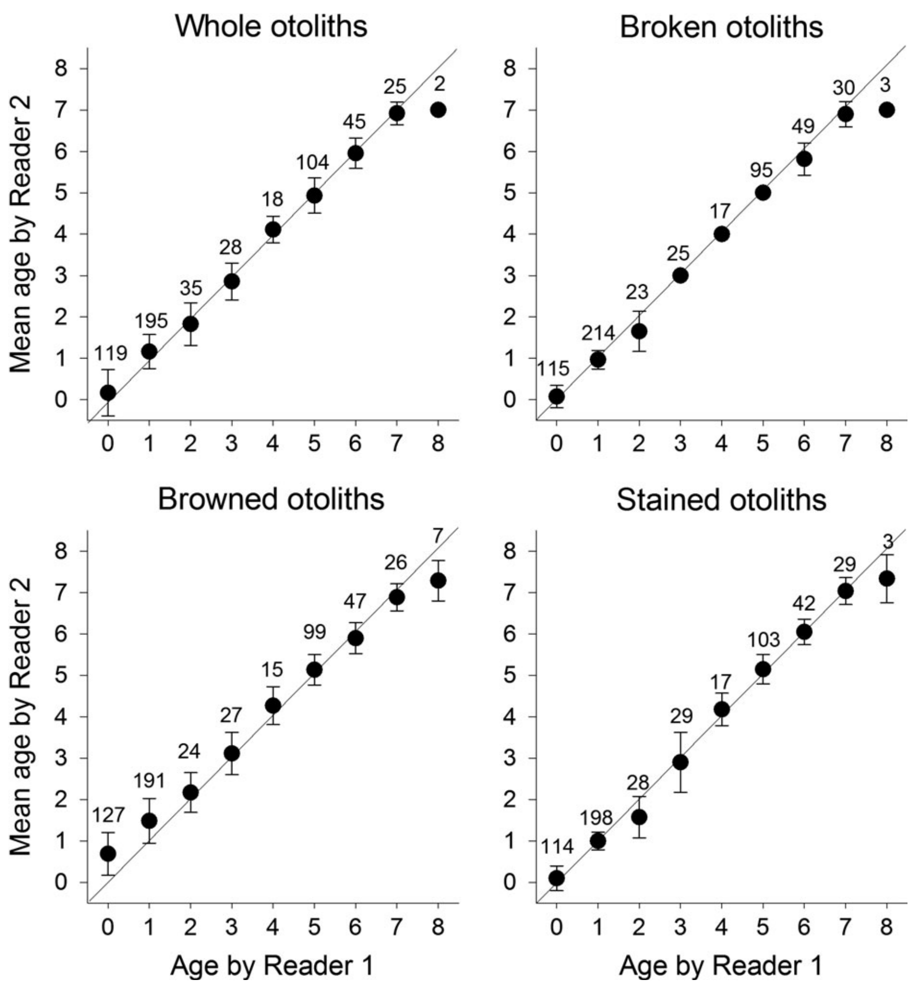


FIGURE 3. Age bias plots (mean [±SD] age assigned by reader 2 for each age assigned by reader 1) for each otolith preparation technique for White Perch collected from Sooner Reservoir during 2015–2016. The diagonal line is the 1:1 agreement line. Sample size is given above the error bars.

least biased of the four techniques, with minimal age differences between readers (from −1 to +1), and most (92.5%) were the same between readers, as evidenced by the size of the SD bars (Figure 3). Age differences between readers for whole otoliths ranged from −5 to +4 but were relatively unbiased, with mean points lying along the 1:1 agreement line. Browned otoliths were biased for the youngest fish (ages 0–1) and the oldest fish (age 8). Stained otoliths were relatively unbiased but with variable estimates between readers for each age.

Precision of age estimates differed among otolith techniques ($F_{3,\ 2,266} = 92.78$, $P < 0.01$). The absolute lowest CV (highest precision) was obtained with broken otoliths, but this was not statistically different from the CV of stained otoliths (Figure 4). Precision was the poorest for browned otoliths, which was the only technique to produce CV values greater than 10%. The CV measurements were consistently higher for fish during early life stages (ages 0–2) but generally were lower and more stable thereafter, except for the oldest fish as measured with broken otoliths (Figure 5). Among months, meanCV was lowest in July and highest in April ($F_{10,\ 2,269} = 20.36$, $P < 0.01$; Figure 6). Moreover, error bars around mean CV estimates in July were the narrowest of all the

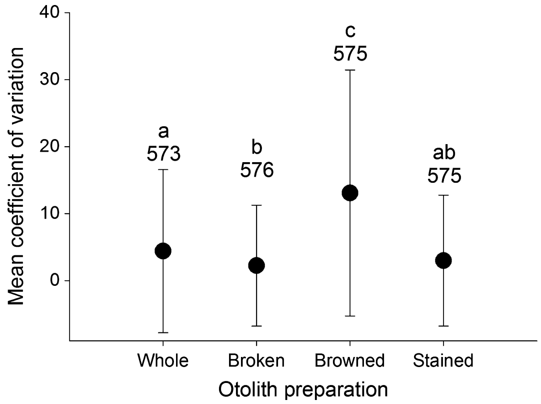


FIGURE 4. Mean (±SD) coefficient of variation from pooled estimated ages across all months for the four otolith preparation techniques used for White Perch. Differences in letters above error bars represent statistically significant differences among techniques. Sample size is given above the error bars.

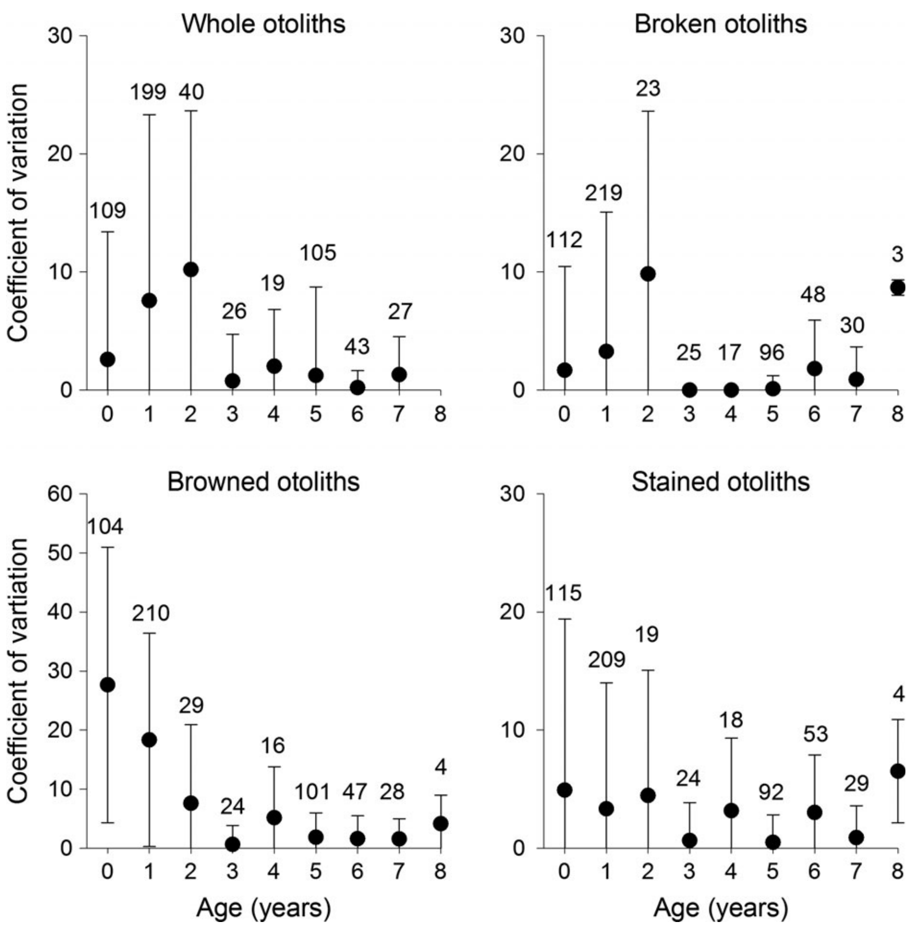


FIGURE 5. Mean (±SD) coefficient of variation (%) for each age-class estimated from the four otolith preparation techniques used to estimate age of White Perch. Sample size is given above the error bars.

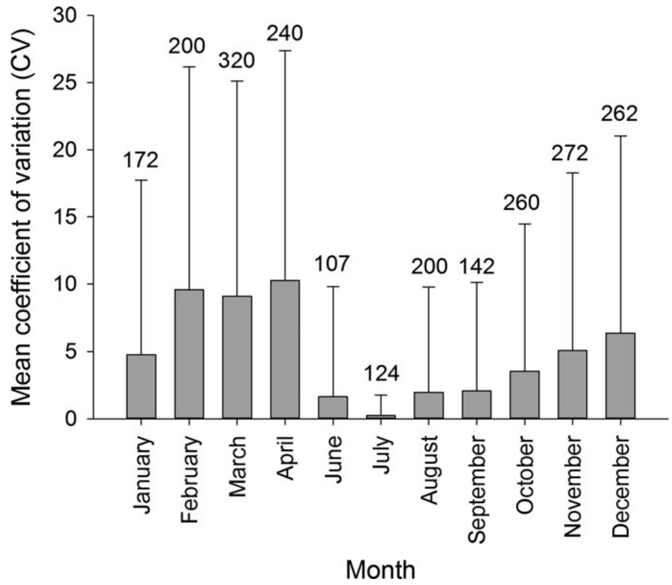


FIGURE 6. Mean (+SD) coefficient of variation (%) of White Perch age estimates by month pooled among four otolith techniques (whole, broken, browned, and stained). Sample size is given above the bars.

months examined, suggesting consistently high precision between readers for every otolith examined.

Using data for July, the month with the highest precision estimates, to construct von Bertalanffy models showed similar performance between models based on whole otoliths and sectioned otoliths (SER = 12.6), which were better than the models produced using browned otoliths and stained otoliths (SER = 18.0; Tables 1, 2; Figure 7). Growth coefficients ($k$) were lower for browned and stained otoliths ($k = 0.35$) compared to whole and broken otoliths ($k = 0.41$). Correspondingly, maximum mean length coefficients ($L$) were larger for techniques that estimated lower $k$-values (e.g., browned otoliths). Predicted length at age had maximum differences in predicted TLs ranging from 1.8 to 8.1 mm TL and CV values less than 4% in all age-classes. The greatest CV differences among predicted TLs occurred for ages 1 and 2.

## DISCUSSION

Browning of otoliths was definitively the worst technique for age precision, whereas whole, broken, and stained otoliths produced roughly similar precision. Plausibly, the smaller otoliths from younger fish were too easily overheated during browning (VanderKooy and Guindon-Tisdel 2003), thus obscuring the annuli rather than enhancing them. Additionally, examination of whole otoliths exhibited a tendency to underestimate age compared to the other techniques, which has been documented for other species (e.g., Largemouth Bass: Buckmeier and Howells 2003; Spotted Bass *Micropterus punctulatus*: Fernando et al. 2014). Whole otoliths also had the largest discrepancies in age estimates, suggesting that other factors made whole otoliths less desirable despite similar precision compared to other methods. Thus, broken and stained otoliths were considered the most similar with regard to precision and age estimation bias, but the extra effort and materials required for staining make this latter technique less desirable to fisheries managers.

Fish estimated to be less than 2 years of age exhibited the lowest precision regardless of technique, which we initially attributed to accessory checks being interpreted as annuli. Checks are typically formed during stressful events (e.g., handling, spawning, low dissolved oxygen, starvation, water temperature, and water level changes; Schramm 1989; Hales and Belk 1992; Jackson et al. 2007; Snow and Long 2016), which can commonly occur in a thermally altered system, albeit probably on a time scale of resolution that we did not measure (e.g., days). For example, the water temperature data we used came from sporadic monitoring over a period of years, which was adequate to describe the whole-lake temperature regime on an annual time scale but inadequate to describe daily or weekly fluctuations. Additionally, we do not know how White Perch in this reservoir respond behaviorally to rapid changes in temperature (e.g., movement) that may induce stress and impart a check on the otolith. We did not attempt to identify and quantify checks specifically, which would require a method to positively identify annuli. Using chemically marked fish, such as

TABLE 1. Estimated von Bertalanffy model parameters (means with SE in parentheses) for White Perch captured from Sooner Reservoir, Oklahoma, in July 2016 ($t_0$ = theoretical age at a length of zero; $k$ = growth coefficient; $L_\infty$ = maximum mean length coefficient). The SE of the regression (SER) is given for each model as an indication of model predictive performance. Fish captured in July provided more precise age estimates than those captured in any other month during the year.

| | Otolith technique | | | |
|---|---|---|---|---|
| Parameter | Whole | Broken | Browned | Stained |
| $t_0$ | −0.665 (0.09) | −0.654 (0.09) | −0.722 (0.15) | −0.722 (0.15) |
| $k$ | 0.405 (0.05) | 0.416 (0.05) | 0.348 (0.06) | 0.348 (0.06) |
| $L_\infty$ | 281.0 (9.44) | 278.1 (8.61) | 290.5 (15.92) | 290.5 (15.92) |
| SER | 12.6 | 12.6 | 18.0 | 18.0 |
| $N$ | 31 | 31 | 31 | 31 |

TABLE 2. Predicted TL (mm) from von Bertalanffy models created for White Perch in Sooner Reservoir using four techniques for estimating age from otoliths (whole, broken, browned, and stained otoliths). The maximum difference (mm) and coefficient of variation (CV) among predicted TLs for each age are given.

| | Predicted TL for each technique | | | | | |
|---|---|---|---|---|---|---|
| Age | Whole | Broken | Browned | Stained | Maximum difference in TL | CV (%) |
| 0 | 66.4 | 66.3 | 64.6 | 64.6 | 1.9 | 1.6 |
| 1 | 137.9 | 138.4 | 131.0 | 131.0 | 7.5 | 3.1 |
| 2 | 185.6 | 186.0 | 177.9 | 177.9 | 8.1 | 2.5 |
| 3 | 217.4 | 217.4 | 211.0 | 211.0 | 6.4 | 1.7 |
| 4 | 238.6 | 238.0 | 234.4 | 234.4 | 4.2 | 1.0 |
| 5 | 252.7 | 251.7 | 250.9 | 250.9 | 1.8 | 0.3 |
| 6 | 262.1 | 260.7 | 262.5 | 262.5 | 1.8 | 0.3 |
| 7 | 268.4 | 266.6 | 270.8 | 270.8 | 4.1 | 0.7 |

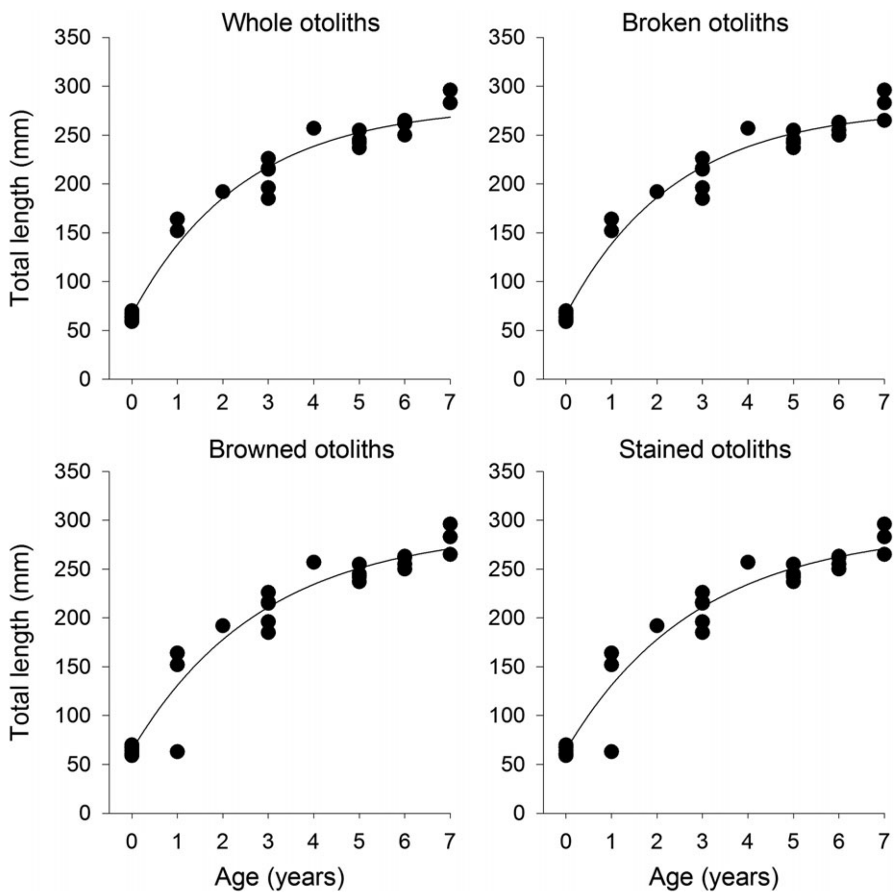


FIGURE 7. Estimated von Bertalanffy growth curves for White Perch collected from Sooner Reservoir in July 2016 (see Table 1 for parameter estimates). Fish captured in July provided more precise age estimates than those captured in any other month during the year.

with oxytetracycline, along with movement data would aid in distinguishing between checks and annuli.

An alternative explanation to check formation that might better explain the low precision of age estimates in young fish is differential growth. At Sooner Reservoir, validated age-1 White Perch ranged in size from about 80 to 240 mm TL (Porta and Snow 2017a), which was the biggest range among all the ages, and diets among these sizes are quite divergent (Porta and Snow 2017b). Diets of White Perch smaller than 130 mm TL in Sooner Reservoir were primarily (99% by weight) composed of zooplankton (46% by weight) and benthic macroinvertebrates (53% by weight), whereas the diets of White Perch between 200 and 249 mm TL were composed of fish prey (45% by weight). Presumably, age-1 White Perch that consume mostly fish are growing faster than age-1 individuals that consume mostly invertebrates, although detailed individual measures of growth would be required to test this. Furthermore, juvenile White Perch exhibit optimum growth at 28.5°C (Kellogg and Gift 1983), which is mostly found in the thermally altered portion of Sooner Reservoir rather than in the areas near the dam, and fish found in these areas may grow differently, which could affect the ability to discern annuli. For example, differences in precision were evident among Bluegill populations along a latitudinal gradient and between quality and stunted populations (both situations presume differences in fish growth; Hoxmeier et al. 2001). As stated earlier, though, individual growth and movement data would be needed to test a hypothesis of location-specific temperature regime on age estimation precision.

Based on results among months, it was clear that July was the optimal time for estimating the age of White Perch in the Sooner Reservoir population. Formation of annuli occurs during April–June in this population (Porta and Snow 2017a), suggesting that the month immediately after annulus formation makes for ideal discrimination. These results are particularly useful because standardized sampling for moronids by the ODWC occurs during experimental gill-net surveys in the fall, when age precision is poor. If obtaining the most precise age estimates is a goal for further study of the White Perch population in Sooner Reservoir, then sampling in July to collect age data specifically would be optimal.

Population-level estimates of fish body growth were definitely affected by the precision of age estimates. The largest differences in predicted TL at age occurred for ages that had the least precise estimates. Additionally, using more precise age estimates for the entire sample produced more precise growth models. Most similar were data from whole otoliths and broken otoliths. Although whole otoliths required no additional preparation and produced models often within 1 mm in predicted TL compared to broken otoliths, whole otoliths also underestimated maximum age (7 years compared to 8 years for all other techniques). Intuitively, additional population-level parameters, such as mortality and recruitment, would be similarly affected, but we did not examine this. The use of broken otoliths was the best method for producing the most precise data overall, particularly from fish captured in July.

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