Comparison of Two Otolith Processing Methods for Estimating Age of Silver Carp

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Abstract: Accurate age estimates are critical in the development, implementation, and assessment of silver carp (*Hypophthalmichthys molitrix*) management plans. Lapilli otoliths are the most commonly used calcified structures for silver carp age estimation, but studies on the precision of two established preparation methods [i.e., grind-and-burn (GB), thin-section (TS)] are lacking. Therefore, we assessed within-reader, between-reader, and between-method precision for 125 silver carp collected from six rivers throughout the Lower Mississippi River Basin (Arkansas, Cache, Mississippi, St. Francis, White, and Yazoo). Additionally, we compared the effort and material costs associated with each method. Overall, younger ages were estimated with the GB method (median estimated age = 6 yr, range = 3–12) than the TS method (median estimated age = 7 yr, range = 3–13). Between-method comparisons revealed low agreement (average CV = 16.40) and significant bias (Evans-Hoenig $\chi^2 = 31.81$, $P < 0.01$) between the two methods, particularly in older individuals. The TS method (average CV = 12.50) displayed similar between-reader precision to the GB method (average CV = 11.75). Younger age estimates for the GB method may be a result of misidentification of annuli near the otolith margin as both readers reported that TS otoliths offered clearer views than GB otoliths. Processing effort (TS method = 6.7 min otolith^{-1}; GB method = 4.6 min otolith^{-1}) and material costs (TS method = US\$0.37 otolith^{-1}; GB method = \$0.34 otolith^{-1}) were similar for the two methods and are likely not a factor when choosing an age estimation protocol. Our results indicate that use of the TS method for silver carp age estimation may lead to less biased age estimates, especially in established populations with greater abundances of older individuals, assuming putative additional annuli observed in thin-sections are true annuli.

Keywords: precision, lapilli, reader agreement, *Hypophthalmichthys molitrix*

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Age estimation is an essential aspect of fish population assessment with direct implications for management (Kerns and Lombardi-Carlson 2017). Fish ages estimated from calcified structures are often used to estimate population parameters such as growth and mortality, and play important roles in understanding processes such as maturation schedules (Olsen et al. 2004, Gobin et al. 2021), recruitment dynamics (Maceina 1997, Yule et al. 2008), and movement patterns (Poole and Reynolds 1996, Crozier and Hutchings 2014). Conversely, the inability to accurately estimate fish age can result in mismanagement of a fishery. For example, if ages for a population are systematically overestimated, mortality will be underestimated and may lead to incorrect management actions (Yule et al. 2008, Hamel et al. 2016). Therefore, emphasis should always be placed on obtaining accurate, precise, and unbiased age estimates before implementing management actions. This is especially challenging when age estimation procedures have not been developed for the species of interest.

Silver carp (*Hypophthalmichthys molitrix*) escaped aquaculture ponds in Arkansas during the 1970s and have invaded much of the Mississippi River Basin (Kolar et al. 2007). Resource managers are particularly concerned about silver carp due to their ability to alter aquatic communities (Sampson et al. 2009, Solomon et al. 2016, Harris et al. 2022), negatively impact native fishes (Irons et al. 2007, Pendleton et al. 2017, Chick et al. 2020), and even injure boaters (Vetter et al. 2015, Spacapan et al. 2016). Therefore, managing silver carp populations has become a high priority, primarily through targeted removal programs (Seibert et al. 2015). However, effective assessment of program outcomes relies heavily on

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population parameters calculated from age estimates. For example, if inaccurate ages lead to incorrect assumptions of life history (i.e., faster growth, higher natural mortality, lower age at maturity, higher fecundity than actually occurring), agency personnel might conclude that targeted removal would be an ineffective control strategy (Klein et al. 2023, Sammons et al. 2023) and focus their efforts elsewhere (Vander Zanden and Olden 2008).

The accuracy of silver carp age estimates remains unknown given that no structure or processing method has been validated (Spurgeon et al. 2015). Additionally, since precise age estimates can still be inaccurate (i.e., precisely wrong, or biased, sensu Campana 2001), measures of precision cannot substitute for measures of accuracy. If the accuracy of estimated ages is unknown or questionable, however, high-precision protocols are preferred over low-precision protocols (Campana et al. 1995). Seibert and Phelps (2013) found that for silver carp, lapilli otoliths provided more precise age estimates compared to postcleithra, pectoral fin rays, and vertebrae. These findings were consistent with studies of related species, such as common carp (*Cyprinus carpio*; Phelps et al. 2007) and numerous other cyprinids (Hawkins et al. 2004, Quist et al. 2007, Phelps et al. 2017). The literature, however, highlights two different otolith processing techniques commonly used for silver carp age estimation – the "thin-section" method (TS method; Hayer et al. 2014, Sullivan et al. 2020, Werner et al. 2022) and the "grind-and-burn" method (GB method; Seibert et al. 2015, Ridgeway and Bettoli 2017, Tripp and Phelps 2018). Comparisons of these two processing methods have shown differences in precision for other species (e.g., Stransky et al. 2005, Edwards et al. 2011, Sakaris and Bonvechio 2020, McKeefry et al. 2023), but this has not been evaluated for silver carp.

Because precise, unbiased age estimates are needed to accurately calculate many population parameters, our first objective was to compare the precision of two processing methods for silver carp otoliths and test for bias within the age estimates. For this objective, precision was defined as the repeatability of an age estimate. Because true bias (i.e., systematic difference in age estimate and true age) could not be determined, bias was defined as a systematic difference between age estimates from the two methods. Given that precision depends not only on the quality of the procedure (i.e., the readability of annual zonation patterns) but also the consistency of the readers, between- and within-reader precision also were compared. Because resource managers often must consider cost and effort associated with age estimation, our second objective was to compare the effort taken to process a set of 10 otoliths and the costs of consumable materials for each processing method.

Methods

Fish Collection

During July–September 2019, silver carp were collected throughout the Lower Mississippi River Basin using daytime, boat-mounted electrofishing (Smith-Root, Vancouver, Washington; pulsed DC, 500V, 60 pulses per second). Rivers sampled included the Arkansas, Cache, Mississippi, St. Francis, White, and Yazoo rivers. Site selection was based on macrohabitat availability (e.g., river side-channels, sandbars, and backwater areas; DeGrandchamp et al. 2008) that were situated near river access sites. Upon capture, total length (TL, mm), weight (g), and sex were recorded for each specimen. Lapilli otoliths were extracted by incision between the preopercle and opercle bones using a reciprocating saw and forceps (Seibert and Phelps 2013). Upon extraction, otoliths were thoroughly cleaned using water and paper towels to remove any residual tissue (Secor et al. 1992) and placed into coin envelopes. Otoliths were allowed to air dry for a minimum of 1 wk before processing (Long and Grabowski 2017). Fish collection was performed under University of Arkansas at Pine Bluff Institutional Animal Care and Use Committee guidelines (id# UAPB2018-05).

Otolith Processing

One otolith from each pair was arbitrarily selected and processed via the GB method as described in Seibert and Phelps (2013). Each reader processed approximately half of the GB otoliths. The anterior side of each otolith was ground using a sequential series of wetted 600-grit and 2,000-grit sandpaper to reveal the nucleus. Otoliths were polished using diamond lapping film to increase clarity. After grinding and polishing, otoliths were heated ground side (i.e., anterior side) down on a hotplate to increase zonation of annuli (Seibert and Phelps 2013, Long and Grabowski 2017). A hotplate was used instead of an open flame to reduce the likelihood of "over-burnt" otoliths (McKeefry et al. 2023). After heating, each otolith was placed posterior side down in putty and submerged in immersion oil (Resolve™ low viscosity immersion oil, Richard Allan Scientific, Kalamazoo, Michigan). A dissecting microscope (Leica MZ95, Leica Microsystems GmbH, Wetzlar, Germany) was used to view GB otoliths using reflected light via a fiber-optic light cable. The orientation of the otolith and the fiber-optic light cable were adjusted for each otolith to optimize readability. Each otolith was imaged using a camera affixed to the microscope and interfaced to a desktop computer (resolution: 1280×1080 pixels; SPOT Idea CMOS Microscope Camera, Diagnostic Instruments, Sterling Heights, Michigan). Digital images were obtained using SPOT Advanced imaging software (Diagnostic Instruments). All steps of the GB method were timed for a subset of 10 otoliths to estimate effort associated with the processing technique.

The second otolith from each pair was processed via the TS method as described in Sullivan et al. (2020). Each reader processed approximately half of the TS otoliths. A clear, cold-setting embedding resin was mixed with slow hardener at a 25:3 ratio by weight (Epofix™, Electron Microscopy Sciences, Hatfield, Pennsylvania). The resin-hardener mix was applied to form a base layer in the wells of plastic embedding molds (multi-well embedding mold, 0.63-cm × 1.27-cm wells, Electron Microscopy Sciences). The base layer was allowed to cure until the epoxy became tacky (approximately 30 min). Each otolith was placed concave side down on the base layer of resin and covered with a top layer of resin, following the same mixing protocol (25:3 resin:hardener ratio by weight). Resin was allowed to cure for a minimum of 24 h before sectioning. After curing, an approximately 0.8-mm thick section was removed from each otolith by cutting along the transverse plane through the otolith core using a low-speed saw (Isomet® 1,000 Precision Saw, Buehler, Lake Bluff, Illinois). Sections were first polished with wetted 2,000-grit sandpaper and then diamond lapping film to increase clarity. After polishing, each otolith was placed on a clear, glass microscope slide with a drop of immersion oil (Resolve™ low viscosity immersion oil, Richard Allan Scientific) and viewed under a compound microscope (BX53M, Olympus, Center Valley, Pennsylvania) using transmitted light. Otolith orientation and brightness were adjusted for each otolith to optimize readability. Each otolith was imaged using a camera (resolution: 1600 × 1200 pixels; DP21, Olympus) affixed to the microscope and interfaced to a desktop computer. Digital images were obtained using cellSens imaging software (cellSens Standard, Olympus). All steps of the TS method were timed for a subset of 10 otoliths to estimate effort associated with this technique.

Age Determination and Precision

Two readers estimated age twice independently for each imaged otolith (i.e., two estimates per reader per image) by recording the number of opaque bands (i.e., white bands under reflected light [GB method], dark bands under transmitted light [TS method]). Readers estimated age without knowledge of fish length or ages previously assigned by themselves or the other reader. Furthermore, to ensure independence, samples were randomized and neither reader estimated both ages for an image on the same day. Because fish were collected in middle to late summer, the outer edge of the otolith was not considered as an annulus (Vilizzi and Walker 1999, Scarnecchia et al. 2006). To obtain a consensus age for each image, a concert read was performed and an agreeable age estimate was determined without knowledge of the paired estimate from the other technique or fish length.

Precision was analyzed across three dimensions: within reader, between readers, and between methods. Within-reader precision represented how often the two age estimates from a single reader agreed for each method. To examine within-reader precision, the two age estimates from each reader for each method were compared. Between-method precision represented similarity between consensus age estimates from the two methods. Between-reader precision (agreement in age estimates between readers) for each method was examined by comparing the four sets of age estimates (two sets of age estimates from each reader).

Each dimension of precision was examined using three different approaches: qualitative evaluation of raw data plots, precision indices, and symmetry testing (McBride 2015, Ogle 2016). Qualitative evaluation of raw data plots was conducted using age-bias graphs, allowing the visual identification of systematic differences (i.e., bias) between two sets of age estimates (Campana et al. 1995). Average coefficient of variation (ACV; Chang 1982, Campana et al. 1995) was used to quantify the similarity (i.e., precision) of different sets of age estimates (Beamish and Fournier 1981). Two other measures of precision, exact agreement rate and rate of agreement within 1 yr, are also reported for each comparison (Campana 2001). Symmetry testing examines systematic bias as deviations in symmetry from the diagonal agreement line in age-agreement tables (McBride 2015) and was conducted using Evans-Hoenig symmetry tests (Evans and Hoenig 1998). Symmetry testing was restricted to the within-reader and between-method comparisons as Evans-Hoenig symmetry tests require two sets of ages and the between-reader comparison was conducted on four sets of ages. A two-sample Kolmogorov-Smirnov test was used to compare age distributions between the two processing methods (Higgins 2004, Ogle 2016). All analyses were performed using the Fisheries Stock Assessment (FSA) package (Ogle et al. 2021) in Program R (R Core Team 2022) with $\alpha = 0.05$ as the threshold for statistical significance.

Total effort for each method was compared by calculating processing effort per 10-otolith sample, with effort partitioned by the steps of each method. Explicitly, the final cure time (minimum of 24 h) was not included in effort estimation for the TS method because it is not an active effort. In other words, the time associated with this step does not require the presence of agency personnel or researchers and may be used to process or estimate age from other trays of otoliths, or focus on other, unrelated tasks. The costs of consumable materials per otolith for each method also were compared, but not non-consumable materials, such as microscopes, low-speed saws, and hotplates. Item costs were obtained from vendor websites. Cost per otolith was calculated by estimating the number of otoliths that could be processed by each item. Where possible, estimates for items were calculated volumetrically (i.e., cutting fluid, epoxy, immersion oil), while others were approximated from the amount of material used during the processing of the otoliths reported in precision comparisons (i.e., sandpaper, lapping film).

Results

A total of 125 silver carp (median TL = 805 mm, range = 427–1025 mm) were collected for age analyses. Approximately equal numbers of silver carp were collected from each river (median = 20 individuals, range 18–24). Within-reader bias was not observed for Reader 2 in either method but was present in both methods for Reader 1 (Table 1; Figure 1). Reader 2 was more precise than Reader 1 for both the TS method and GB method (Table 1; Figure 1). However, within-reader bias showed no trends with fish age (Table 1; Figure 1).

Between-reader comparisons yielded similar levels of precision for each method (Table 1; Figures 2 and 3). However, between-method comparisons revealed significant bias and little agreement between the two processing methods (Table 1; Figure 4). Age estimate distributions significantly differed between the two methods

Table 1. Measures of precision and bias of silver carp age for within-reader, between-reader, and between-method comparisons using otoliths from 125 silver carp processed via the thin-section (TS) and grind-and-burn (GB) methods. Note: between-reader comparisons were based on more than two sets of age estimates and measures of bias (i.e., Evans-Hoenig symmetry testing) could not be calculated for those comparisons.

	Within Reader 1		Within Reader 2		Between Readers		
Statistic	TS	GB	TS	GB	TS	GB	Between Methods
Average CV (ACV)	14.06	9.64	5.71	7.58	12.50	11.75	16.40
Evans-Hoenig χ^2	17.60	15.69	2.29	4.09			31.81
Evans-Hoenig *P*	< 0.01	< 0.01	0.52	0.25			< 0.01
Exact agreement (%)	20.8	40.8	53.6	48.0	7.0	12.0	22.4
Agreement within 1 yr (%)	73.6	85.6	93.6	93.6	55.0	66.0	62.4

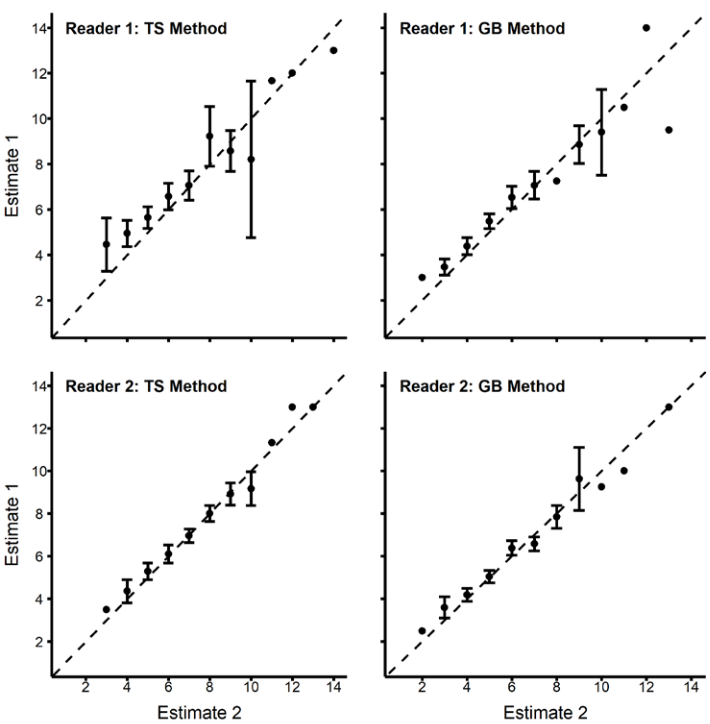

Figure 1. Age-bias graphs for each of the four pairwise, within-reader comparisons where readers used otoliths to estimate the age (yr) of silver carp. Error bars represent the 95% confidence interval about the mean age estimate assigned during the first read for all fish assigned an estimated age during the second read. Dashed line represents a 1:1 relationship. Note: error bars are not shown if fewer than five individuals were estimated of a given age.

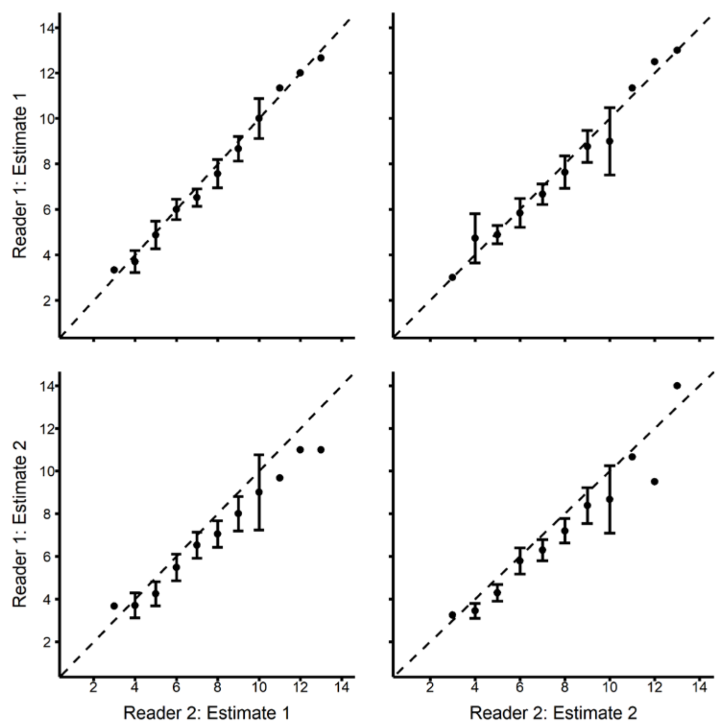

Figure 2. Age-bias graphs for each of the four pairwise comparisons to assess between-reader precision using otoliths processed with the thin-section method. Error bars represent the 95% CI about the mean age (yr) estimate assigned during the first read for all silver carp assigned an estimated age during the second read. Dashed line represents a 1:1 relationship. Note: error bars are not shown if fewer than five individuals were estimated of a given age.

(two-sample K-S test, $D = 0.24$, $P \leq 0.01$) with the TS method ranging from 3–13 yr (median = 7) and the GB method ranging from 3–12 yr (median = 6; Figure 5). Processing effort was 46% greater for the TS method (6.7 min otolith^{-1}) than the GB method (4.6 min otolith^{-1}; Table 2), but consumable costs were relatively similar on a per otolith basis for both methods (Table 3).

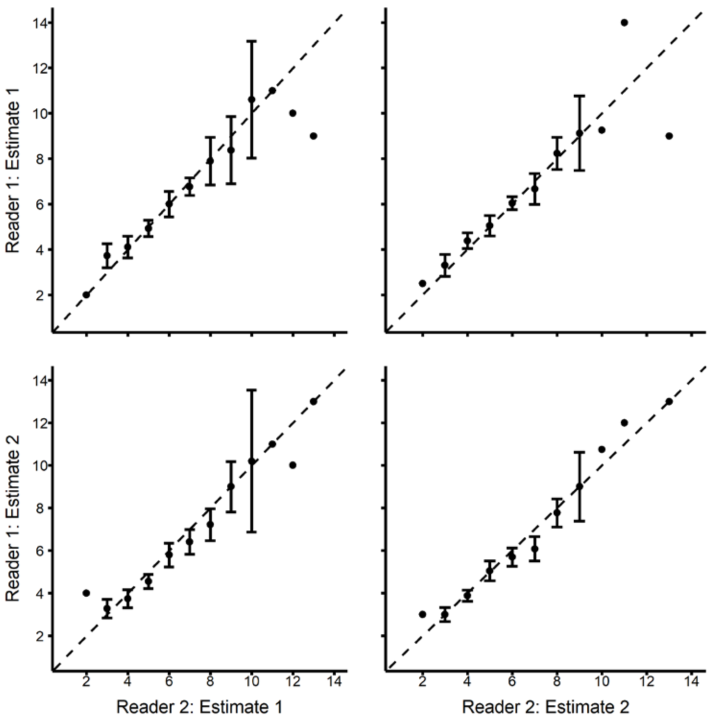

Figure 3. Age-bias graphs for each of the four pairwise comparisons to assess between-reader precision using otoliths processed with the grind-and-burn method. Error bars represent the 95% CI about the mean age (yr) estimate assigned during the first read for all silver carp assigned an estimated age during the second read. Dashed line represents a 1:1 relationship. Note: error bars are not shown if fewer than five individuals were estimated of a given age.

Table 2. A comparison of effort to perform the thin-section and grind-and-burn otolith processing techniques (per 10 otoliths).

Thin-Section Method		Grind-and-Burn Method	
Step	**Effort (min per 10 otoliths)**	**Step**	**Effort (min per 10 otoliths)**
Mixing epoxy (bottom layer)	2	Otolith grinding	18
Applying epoxy (bottom layer)	3	Otolith polishing	8
Placing otoliths in mold	4	Otolith burning	20
Bottom layer of epoxy to begin curing	30		
Mixing epoxy (top layer)	2		
Applying epoxy (top layer)	3		
Sectioning otoliths	23		
Total	67 min	**Total**	46 min
Per otolith	6.7 min	**Per otolith**	4.6 min

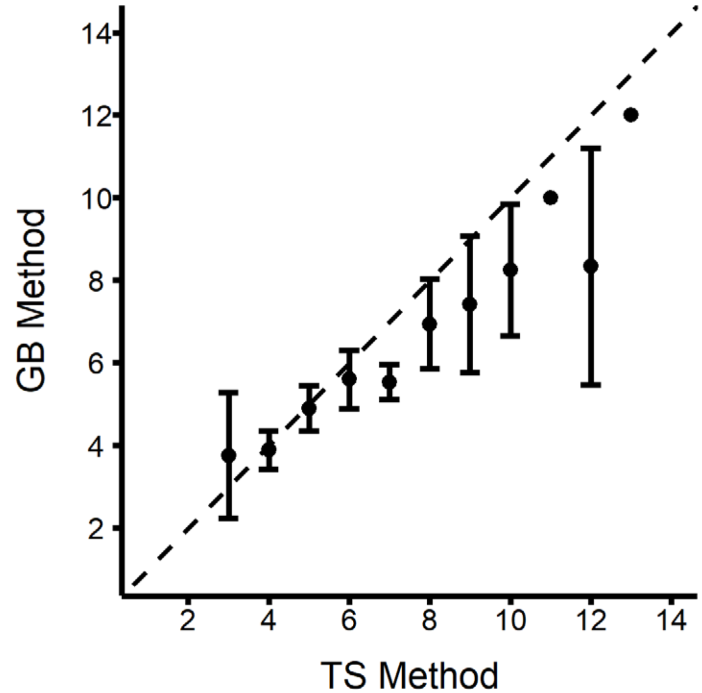

Figure 4. Age-bias graph comparing estimates from two otolith processing methods. Error bars represent the 95% CI about the mean age (yr) estimate assigned during the first read for all silver carp assigned an estimated age during the second read. Dashed line represents a 1:1 relationship. Note: error bars are not shown if fewer than five individuals were estimated of a given age.

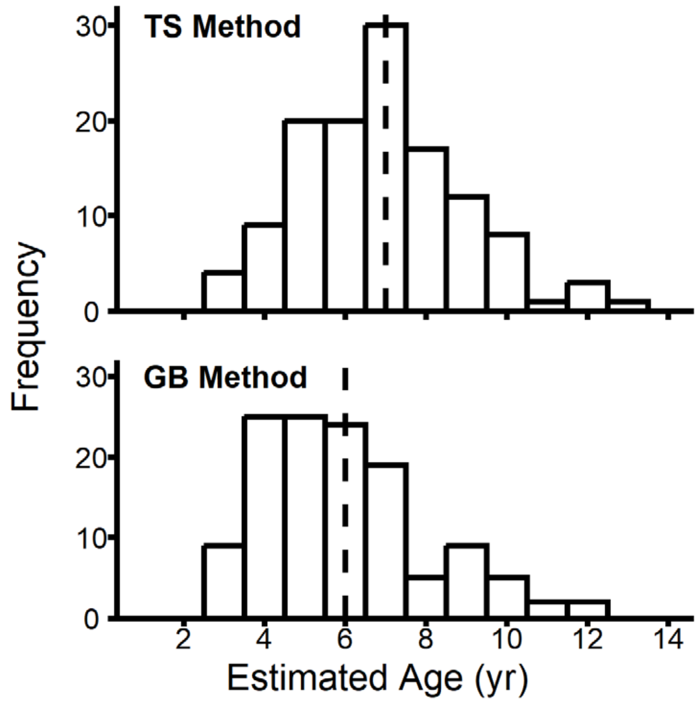

Figure 5. Age estimate distributions for otoliths from 125 silver carp processed via the thin-section method and grind-and-burn method. Median estimated age is represented by a dashed line.

Table 3. Estimated costs (US$) of consumable materials required for the grind-and-burn and thin-section otolith processing methods. These materials can be obtained from various vendors; thus, less-expensive options may be available.

Thin-Section Method				Grind-and-Burn Method			
Item	Item cost	Estimated otoliths	Cost per otolith	Item	Item cost	Estimated otoliths	Cost per otolith
Epredia Resolve Immersion Oil (M3000)	$28.00	1770	$0.02	Epredia Resolve Immersion Oil (M3000)	$28.00	885	$0.03
2000-grit wet/dry sandpaper (10-pack)	$7.98	500	$0.02	600-grit wet/dry sandpaper (10-pack)	$7.98	250	$0.03
Buehler Isocut fluid (0.95 L)	$63.00	4023	$0.02	2000-grit wet/dry sandpaper (10-pack)	$7.98	500	$0.02
EMS diamond lapping film (8″ × N/H PSA)	$32.00	125	$0.26	EMS diamond lapping film (8″ × N/H PSA)	$32.00	125	$0.26
EMS Epofix kit, includes 1 L resin and 130 ml hardener	$190.00	4432	$0.04				
EMS multi-well embedding mold	$12.00	1200	$0.01				
Total	$332.98		$0.37	**Total**	$75.96		$0.34

Discussion

Our between-method comparisons indicated that, on average, age estimates differed between the two methods used to prepare and interpret otoliths. On average, the GB method produced age estimates approximately 1 yr younger than the TS method for silver carp. Furthermore, age estimate differences between methods appeared to become more severe with older age classes of silver carp, which has been reported for other species (Stransky et al. 2005, Edwards et al. 2011, McKeefry et al. 2023). For example, Edwards et al. (2011) reported that cracked-and-burned otoliths consistently underestimated age in burbot (*Lota lota*) compared to thin sections. Thin sections also offered better clarity at the otolith margins, which resulted in fewer age discrepancies with older individuals. McKeefry et al. (2023) found that cracked-and-burned otoliths produced significantly younger age estimates than thin-section otoliths for lake whitefish (*Coregonus clupeaformis*) due to clarity at the otolith margin. Interestingly, McKeefry et al. (2023) documented "over-burnt" otoliths, which resulted in the loss of outer annuli. In our study study, different microscopes were used for each processing technique (i.e., compound microscope for the TS method; dissecting microscope for the GB method), following typical protocols for each method (Quist and Isermann 2017). Thus, increased clarity at the otolith margin in TS otoliths may have been influenced by microscope type, in addition to processing technique. Furthermore, ages were estimated from photos in an effort to minimize the variability introduced by differential placement of side illumination in the GB method (Stransky et al. 2005). Restricting the light angle to a fixed point could have artificially lowered the age estimates in the GB method, as changing the angle of illumination can increase discernability in outer annuli (Sakaris and Bonvechio 2020). Nonetheless, the TS method allowed readers to better discern annuli at the otolith margin and distinguish the otolith edge with the outermost annuli, thus, eliminating the "edge effect" (Frommel et al. 2021).

Within- and between-reader comparisons revealed generally low precision in both processing methods, suggesting silver carp age estimation may be relatively difficult. This general finding has been supported by previous work (e.g., Kolar et al. 2007, Hayer et al. 2014). However, currently no single structure and preparation method has been validated for this species. Therefore, multiple structures and procedures have been used to estimate silver carp age with varying levels of success. Broaddus and Lamer (2022) reported 70.3% exact between-structure agreement among pectoral ray, postcleithra, and vertebrae from silver carp collected in the Upper Mississippi River. Lebeda (2020) found that silver carp ages estimated from pectoral fin-ray sections in Kentucky Lake had an exact between-reader agreement of 87% but were considerably less precise for fish older than 6 yr. Fernholz (2018) noted that sectioned lapilli otoliths resulted in an exact agreement of only 31% for silver carp in the Tennessee and Cumberland rivers. For silver carp collected from the Illinois River, Morgeson (2015) noted difficulty estimating age and low between-reader precision from sagittal otoliths and postcleithrum. In a review of 117 studies referencing age estimation precision, Campana (2001) found a median CV value of 7.6% for between-reader comparisons, which is much lower than most reported values from silver carp age estimation studies, including ours. One possible explanation of lower precision in silver carp age estimation is their fast growth rate, which can result in faint annuli that are easily overlooked (Kowalewski et al. 2012). When combined with the high longevity of many populations (i.e., up to 15 age classes) that results in crowding at the otolith margin in older individuals, overall difficulty increases. Additionally, false annuli (i.e., checks; Buckmeier et al. 2012) are commonly observed during silver carp age estimation. In this study, lower within- and between-reader precision relative to other species (i.e., Campana 2001), could have been a result of these issues.

The relative difficulty of silver carp age estimation may necessitate more intensive training programs than required for other species. In this study, both readers were moderately experienced (3–5 yr) in estimating age from otoliths in other species (e.g., bluegill [*Lepomis macrochirus*], smallmouth bass [*Micropterus dolomieu*], and walleye [*Sander vitreus*]) but were relatively new to silver carp age estimation (1–2 yr). As such, before age estimation began, both readers defined criteria for identifying annuli by using the available published and gray literature. Still, the relatively low precision in this study could be a result of reader experience and inadequate training. Reader experience has been shown to be positively associated with the precision of age estimations (Campana and Moksness 1991, Campana 2001, Rude et al. 2013, McKeefry et al. 2023). This trend can be attributed to increased pattern recognition in more experienced readers (Morison et al. 2005). Therefore, to alleviate this issue, management agencies and researchers conducting silver carp age estimation should develop thorough training programs and consider increasing the minimum number of training otoliths required for new readers (e.g., Morison et al. 2005).

The TS method required approximately 50% more time effort per otolith than the GB method for silver carp age estimation. Edwards et al. (2011) reported that the section method required considerably greater effort to process otoliths than the crack-and-burn method. Sakaris and Bonvechio (2020) found that total expended effort, including time taken to estimate age, also was greater with the cut method than the ground method for blue catfish (*Ictalurus furcatus*) and channel catfish (*I. punctatus*), but less than the ground method for flathead catfish (*Pylodictis olivaris*). Sakaris and Bonvechio (2020) noted the cut method was likely quicker than the ground method for flathead catfish because the population sampled contained a larger proportion of older individuals with larger, thicker otoliths. Studies reporting processing times, including Edwards et al. (2011) and Sakaris and Bonvechio (2020), often include the time required to allow the top layer of epoxy to fully cure for the TS method. Since this step does not require active effort (i.e., this step does not require the presence of agency personnel or researchers), removing this down time from the calculation might be more insightful. For example, as the authors of this study waited for the epoxy to begin curing, we would section another batch of otoliths, practically eliminating the down time. In this study, removing this time resulted in minimal differences between the processing times of the two methods.

Total costs of consumable materials were approximately 450% greater for the TS method than the GB method with silver carp. When the number of otoliths that could be processed with the purchased materials was considered, however, the differences between methods were minimal. Similar analyses (e.g., Edwards et al. 2011, Sakaris and Bonvechio 2020) have also reported large differences in the total cost of consumable materials but did not calculate the cost per otolith. Since the true difference in cost between the two methods are relatively small when considered per otolith, cost is probably not an important consideration once the commitment to age fish has been made.

Overall, this study documents relatively small changes in age estimation protocol can result in different age estimates in silver carp. Furthermore, structure processing protocol might be equally important as structure selection for silver carp age estimation given that differences in precision were similar between the two processing methods compared in this study and the four structures compared in Seibert and Phelps (2013). Still, this study did not use known-age fish, so further work is needed to confirm that the additional annuli observed in thin-sections represent true annual increments. Additionally, relatively low precision in within- and between-reader comparisons highlights the need for known-age structures to aid in training and quality control programs specific to silver carp age estimation. Given that silver carp age estimation studies are relatively new, there are few resources currently available to researchers and biologists to create these programs. Cost and effort are similar for both methods and likely not an important factor when selecting a silver carp otolith processing protocol. Our results indicate that use of the TS method for silver carp age estimation may lead to less biased age estimates, especially in established populations with greater abundances of older individuals, assuming putative additional annuli observed in thin-sections are true annuli.

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