Comparison of Browned and Standard Otolith Preparation Methods for Estimating Age of Catfish in Oklahoma

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Abstract: Catfish are highly regarded by recreational anglers as sportfish in some areas of North America and are intensively managed by fisheries biologists. Accurate population metrics (e.g., growth, mortality, recruitment, age, and size at maturity) are essential to manage these fisheries, which relies on accurate age estimates for fish in these populations. When otoliths are used for age estimation, they are typically sectioned or ground in a transverse plane, but otolith preparation prior to sectioning may differ. Browning otoliths prior to sectioning to help distinguish annuli has been used by some biologists, but there is a need to determine if this technique results in increased precision. Browning otoliths substantially increases otolith processing time; thus, it should only be done if it demonstrably increases aging precision. The objective of this study was, therefore, to compare precision of age estimates between browned and standard otoliths for black bullhead (*Ameiurus melas*), blue catfish (*Ictalurus furcatus*), channel catfish (*I. punctatus*), flathead catfish (*Pylodictis olivaris*), and yellow bullhead (*A. natalis*). Paired *t*-tests were used to compare consensus ages and CV of reader age estimates between aging techniques. Consensus age estimates were lower in browned otoliths compared to standard otoliths only for black bullhead and yellow bullhead. Reader CV was lower for standard otoliths for black bullhead and higher for yellow bullhead but was similar between techniques for the other species. The results of this study suggested that only otoliths from yellow bullhead need to be browned, and managers may be able to forego this lengthy process while still achieving precise age estimations for most catfish species.

Key words: Ictaluridae, lapillus, preparation, age estimates, precision

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Catfish are popular sportfish pursued by recreational anglers in many areas of the United States due to their potential to reach trophy sizes, fighting characteristics, and food quality (Prather 1959, Moss and Tucker 1988, Graham 1999, Wilde and Ditton 1999, Arterburn et al. 2002, Bodine et al. 2013). For these reasons, biologists and anglers desire management efforts to ensure these populations are self-sustaining to produce and maintain trophy fisheries (Arterburn et al. 2002, Boxrucker and Kuklinski 2006). When managing these fisheries, however, it is necessary to understand their life history and population characteristics, which, in turn, requires reliable age information (Kwak et al. 2006). Therefore, fisheries managers need to determine the aging technique that provides the most accurate and precise age estimates (Crumpton et al. 1984).

The two most commonly used structures for estimating age of catfish are pectoral spines (Mayhew 1969, Turner 1980, Moss and Tucker 1989) and otoliths (Crumpton et al. 1984, Nash and Irwin 1999, Buckmeier et al. 2002, Maceina et al. 2007). Removal of pectoral spines from channel catfish is non-lethal (Stevenson and Day 1987), making this structure appealing for age estimation, which is the most common reason spines are used for age estimation of catfish (Maceina et al. 2007). However, most studies have found that otoliths provide more accurate and precise age estimations for catfish (Nash and Irwin 1999, Buckmeier et al. 2002).

In catfish, the lapillus is the largest otolith and is preferred for estimating ages (Long and Stewart 2010), which usually involves sectioning and grinding in the transverse plane to the point where the nucleus and annuli are visible (Maceina 1988, Nash and Irwin 1999, Buckmeier et al. 2002). A standard method for preparing lapilli otoliths from catfish was described by Buckmeier et al. (2002) where researchers sanded otoliths with 600-grit sandpaper to reveal the nucleus and annuli. In an effort to make annuli more distinguishable, they also burned otoliths prior to sanding by placing them on a hotplate until the otolith turned brown (Buckmeier et al. 2002). Long and Grabowski (2017) also suggested that burning otoliths can help increase contrast between accretion and discontinuous zones of the otolith.

Some subsequent studies have browned otoliths prior to estimating ages of catfish (e.g., Mauck and Boxrucker 2004, Boxrucker and Kuklinski 2006, Kwak et al. 2006, Colombo et al. 2010, Snow et al. 2017), but other studies have not (Stewart et al. 2009). Browning of catfish otoliths is the standard procedure for the Oklahoma Department of Wildlife Conservation (ODWC), but this adds a substantial amount of processing time. Because a large number of otoliths are processed annually by ODWC staff, we wanted to ensure that otolith browning is necessary. Therefore, the objective of this study was to compare aging precision and processing time between browned and standard otoliths for five species of catfish commonly found in Oklahoma.

Table 1. Bodies of water where catfish were collected. Sample size (*n*), range of TL (mm), and range of age estimates (years) are given.

	Name	Species	*n*	TL (mm)	Age (yrs)
Lake	Carl Blackwell	Flathead catfish	11	686–1092	8–28
	Carl Etling	Black bullhead	334	90–356	0–9
	Elmer	Black bullhead	18	204–377	1–5
	Evan Chambers	Channel catfish	122	208–531	2–18
	Hugo	Blue catfish	25	229–595	2–15
	Kaw	Blue catfish	39	235–775	2–18
	Meeker	Blue catfish	101	90–451	0–15
	New Spiro	Yellow bullhead	1	262	1
	Prague	Yellow bullhead	26	201–335	1–5
	Stillwell	Yellow bullhead	39	174–339	1–11
	Tecumseh	Black bullhead	5	202–260	2–5
	Thunderbird	Channel catfish	113	200–656	1–14
	Thunderbird	Flathead catfish	54	137–860	0–19
River	Arkansas	Blue catfish	102	176–1050	2–23
	Kiamichi	Blue catfish	101	192–843	1–12
	Red	Blue catfish	76	229–701	1–15

Methods

Blue catfish (*Ictalurus furcatus*; $n = 496$), channel catfish (*I. punctatus*; $n = 276$), flathead catfish (*Pylodictis olivaris*; $n = 77$), black bullhead (*Ameiurus melas*; $n = 380$), and yellow bullhead (*A. natalis*; $n = 68$) were collected using a variety of sampling gears for age estimation. These fishes were collected from 16 different water bodies throughout Oklahoma over a 13-year period (2004–2017; Table 1). Upon collection, fish were measured (TL; mm) and lapilli were extracted from each fish (Long and Stewart 2010). Once removed, both otoliths were cleaned and placed into an individually numbered envelope and left to dry for at least 24 h prior to processing (Secor et al. 1992, Snow et al. 2017). One otolith from each fish was browned by placing it on a hot plate (Model HPA1915B, Barnstead/Thermolyne, Dubuque, Iowa) at 104° C until the otolith turned brown. Both otoliths (standard and browned) were then placed into one of 21 individual cells of a silicon mold (Electron Microscopy Sciences, Fort. Washington, Pennsylvania) and covered with epoxy for mounting (West System 205-B hardener and 105-B Epoxy resin, Gougeon Brothers Inc., Bay City, Michigan; Sakaris et al. 2017). Otoliths were oriented with the anterior-posterior axis parallel to the long axis of the rectangular mold with the distal side of the otolith facing up. Once the epoxy cured, all otoliths were cut in a transverse plane (Secor et al. 1992) with a low-speed IsoMet saw (Buehler Model 11-1280-160 using a 127-mm diameter x 0.4-mm thickness blade; Lake Bluff, Illinois) and viewed in cross section.

Otoliths were placed cut-side up in a dish containing black modeling clay, submerged in water, and viewed with a dissecting microscope (4x–90x) using a fiber optic filament attached to a light source to illuminate annuli (Buckmeier et al. 2002, Snow et al. 2017). Otoliths were viewed before polishing to determine the location of the nucleus relative to where the cut was made. This helped determine the amount of polishing needed following sectioning. Otoliths were then polished with 2000-grit sandpaper until the nucleus and annuli became clear and distinguishable. After polishing to desired clarity, otoliths were placed back into envelopes and samples were read in random order. Each otolith was examined independently by two readers to estimate the age of each fish. If the readers disagreed on the number of annuli in an otolith, then that otolith was read again in concert by both readers until an agreement was made. However, if one of the otoliths was deemed unreadable, that individual fish was removed from the study (both the browned and standard otoliths were removed).

Preparation time for both otolith methods (standard and browned) was recorded in minutes. This was achieved by using a timer to record start and stop times for each of the four timed trials for both methods (four replicates). Otoliths from 426 fish were used across the four trials (107 fish per trial on average). For the browned otolith method, the total time needed to remove otoliths from the envelopes, place onto the hotplate until browned, move otoliths from the hotplate to the silicon mold, orient the otoliths, and apply epoxy to the cells was recorded. A similar approach was taken for the standard otolith method where the total time needed to remove otoliths from the envelopes, move to the silicon mold, orient the otoliths, and apply epoxy to the cells was recorded. The total processing time for each otolith preparation method (standard and browned) was recorded in minutes. Upon completion of a trial, the number of otoliths processed was divided by the time (min) to achieve number of otoliths min^{-1}. A two- sample *t*-test was calculated to compare processing rates between otolith preparation methods.

Mean CV and percent agreement were calculated to measure between reader precision for age estimates from browned and standard

Table 2. Mean between-reader CV of each aging method and outcomes of paired t-test examining CV differences between methods for each species. Asterisk denotes significance ($P < 0.05$).

Species	*n*	Browned (CV)	Standard (CV)	*t*	df	*P*
Black bullhead	357	12.39	8.17	2.58	356	0.01*
Blue catfish	444	1.73	2.21	−1.61	443	0.06
Channel catfish	234	4.16	3.04	1.44	233	0.08
Flathead catfish	66	12.55	9.07	0.88	65	0.19
Yellow bullhead	66	2.04	10.23	−4.59	65	0.00*

Table 3. Mean consensus age estimates of each aging method and outcomes of paired *t*-test examining age differences between methods for each species. Asterisk denotes significance ($P < 0.05$).

Species	*n*	Browned (Mean Age)	Standard (Mean Age)	*t*	df	*P*
Black bullhead	357	3.50	3.62	−3.83	356	0.01*
Blue catfish	444	7.16	7.23	−2.27	443	0.01*
Channel catfish	234	5.75	5.76	−0.18	233	0.43
Flathead catfish	66	7.53	7.76	−1.84	65	0.04*
Yellow bullhead	66	2.82	3.30	−5.95	65	0.01*

otoliths for each species (Campana et al. 1995, Campana 2001). The CV of age estimates from browned and standard otoliths for each species was compared with a paired *t*-test (Hurley et al. 2004, Porta et al. 2017). Further, paired *t*-tests were used to compare consensus ages from browned and standard techniques. To visualize bias between readers, age-bias plots were constructed to compare age estimates between readers and to final consensus age estimates for each otolith preparation method and species. Additionally, age-bias plots were used to compare final consensus ages between techniques for each species. The 95% confidence interval of mean age estimates was calculated for each reader (Campana et al. 1995). Paired and two-sample *t*-tests were analyzed using Microsoft Excel. All statistical outcomes were considered significant if $P \leq 0.05$.

Results

A total of 1297 fish were processed and examined, but only 1167 were readable for age estimation (Table 1). Mean between-reader CV was similar between browned and standard otoliths for blue catfish, channel catfish, and flathead catfish, but was 52% higher for browned otoliths of black bullhead (Table 2). Conversely, mean between-reader CV for yellow bullhead was more than five-fold higher for standard otoliths. Mean consensus age was similar between browned and standard otoliths for channel catfish (Table 3). For all other species, mean consensus age was lower for browned otoliths compared to standard otoliths.

Agreement between readers for blue catfish, channel catfish, and black bullhead were similar between techniques, ranging from 78%–84% (browned) and 75%–83% (standard). Readers agreed within one year more than 90% of the time using either method across species, except for browned otoliths of channel catfish (80%). For yellow bullhead and flathead catfish, agreement was lower for standard otoliths (55% and 59%) than browned otoliths (92% and 77%). However, observed agreement within one year for yellow bullhead was 100% for browned otoliths and 90% for standard otoliths. Flathead catfish also had similar agreement within one year for browned otoliths (73%) and standard otoliths (82%).

Although precision was generally high between reader age estimates and final consensus ages for both methods, no consistent reader biases were identified for any of the species (Figure 1, Figure 2). Visual observation of age-bias plots for blue catfish and channel catfish suggested there was no bias between the two techniques even though variability increases with age (Figure 3). Age estimates of flathead catfish using standard otoliths appeared to be slightly higher in younger fish than age estimates from browned otoliths (Figure 3). Age-bias plots for yellow bullhead and black bullhead suggested that age estimates from standard otoliths yielded higher age estimates than estimates from browned otoliths in most age classes (Figure 3), which was consistent with the results of the mean age analysis.

Mean processing rate for standard otoliths was 4.2 otoliths min^{-1} (range = 3.1 to 5.0). This was significantly faster ($t = 7.70$, df = 3, $P = 0.002$) than the mean processing rate for browned otoliths (Figure 4), which was 0.86 otoliths min^{-1} (range = 0.81 to 0.87).

Discussion

Precision was similar between browned and standard otolith age estimates for the three non-bullhead catfish species. For black bullhead, age estimates from browned otoliths were more variable but differed from those from standard otoliths on average by only 0.12 yrs; therefore, standard otoliths could be used to save time. Conversely, age estimates for yellow bullhead using standard otolith methods were much more variable and mean ages differed by almost 0.5 yrs. Species-specific variation in effectiveness of the otolith browning technique has been observed in other studies. Snow et al. (2018) found that browned otoliths were less precise when compared to whole otoliths and stained otoliths for white perch (*Morone americana*). However, browned otoliths of golden redfish (*Sebastes marinus*) produced similar age estimates to those processed using standard methods (Stransky et al. 2005). Barber and McFarlane (1987) found similar age estimates between whole and browned otoliths for arctic char (*Salvelinus alpines*) for fish up to age 8, but age estimates for older fish became highly variable us-

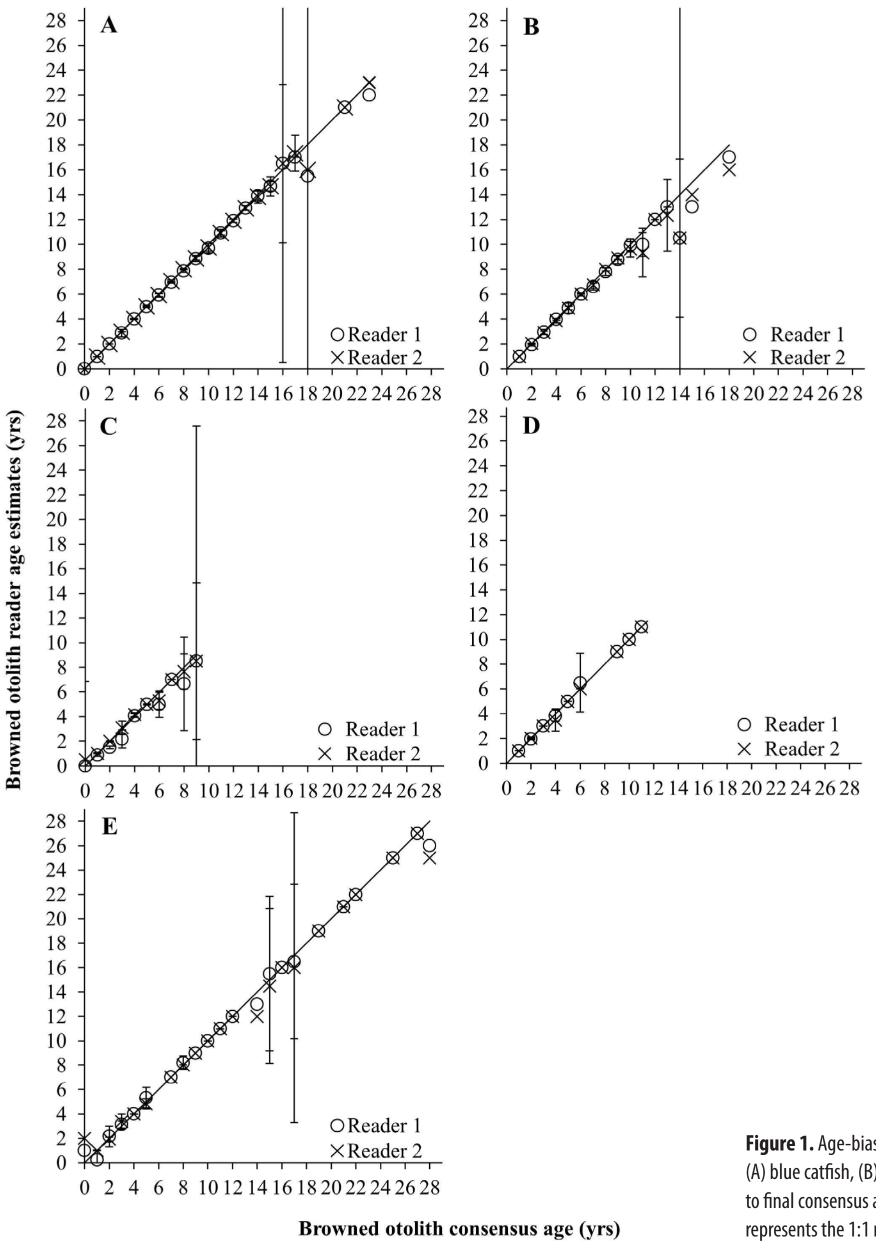

Figure 1. Age-bias plots comparing Reader 1 and Reader 2 age estimates from browned otoliths for (A) blue catfish, (B) channel catfish, (C) brown bullhead, (D) yellow bullhead, and (E) flathead catfish to final consensus age estimates. Error bars represent the 95% confidence interval. The diagonal line represents the 1:1 relationship between consensus ages and reader 1 and 2 estimated ages.

ing either technique. Conversely, Strickland and Middaugh (2015) found that browned otoliths in spotted suckers (*Minytrema melanops*) aided in detecting annuli closer to the nucleus compared to otoliths that were not browned. Based on our results, it appears that only otoliths of yellow bullhead require browning to attain the most precise age estimates.

Readability of an otolith after processing may affect precision more than the otolith aging technique (browned vs standard). In this study, 10% of the fish were excluded because one or both of their otoliths were deemed unreadable. This was usually due to otolith processing error (i.e., otolith was sectioned improperly). When the section is made too close to the posterior end of the otolith, some or all of the annuli will not be distinguishable. Murie et al. (2009) observed this problem when processing lapilli otoliths from yellow bullhead using the diagram described by Buckmeier et al. (2002) that illustrated the correct sectioning plane for channel catfish. This sectioning location, however, is too far posterior on yellow bullhead otoliths. During preparation of lapilli otoliths from the five species of catfish evaluated in this study, we noticed that the nucleus location within the otolith varied among catfish species (Figure 5). Previous studies have also shown that otolith morphology can vary across species (Vanderkooy and Guindon-Tisdel 2003, Popper et al. 2005).

Otoliths were sectioned in the transverse plane using a low-

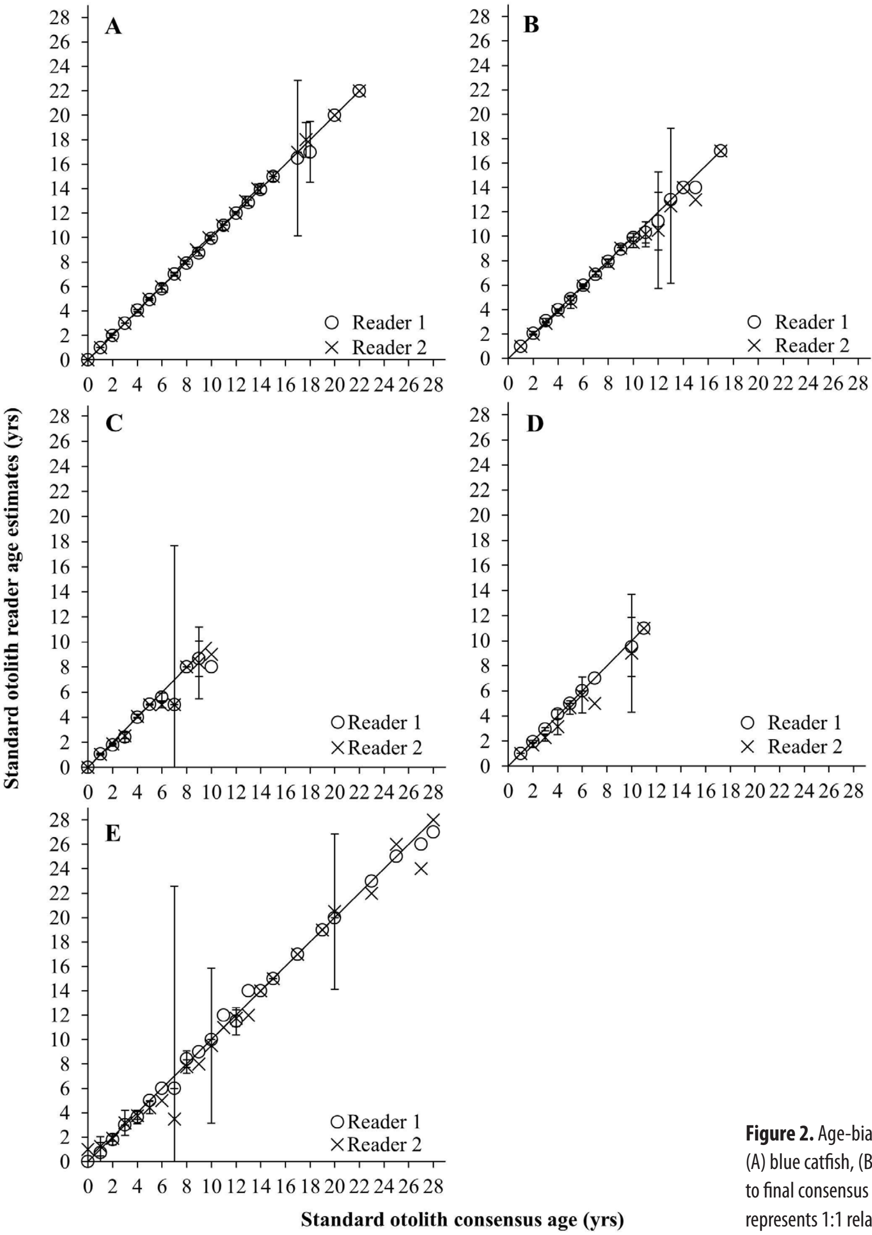

Figure 2. Age-bias plots comparing Reader 1 and Reader 2 age estimates from standard otoliths for (A) blue catfish, (B) channel catfish, (C) brown bullhead, (D) yellow bullhead, and (E) flathead catfish to final consensus age estimates. Error bars represent the 95% confidence interval. The diagonal line represents 1:1 relationship between consensus ages and reader 1 and 2 estimated ages.

speed saw that did not have the option of changing the angle of the cutting blade. After sectioning, some otoliths had to be polished at an angle in order to reveal all annuli. This phenomenon was observed most often in older flathead catfish. In otoliths of flathead catfish age 14 and older, early annuli near the nucleus seemed to be recessed compared to annuli on the distal edge. However, with the use of side illumination using a fiber optic light source, we were able to confirm these less-distinct annuli near the otolith's nucleus and continue polishing until all annuli were clear. Although polishing at the same angle as the otolith was sectioned would reveal the annuli closet to the nucleus, the annuli on the distal edge of the otolith would have been removed. The difficulty of viewing early annuli likely explains why variability between age estimates was greater in older fish, resulting in decreased aging precision.

Some studies have ground otoliths to the nucleus and checked periodically throughout the process to ensure all annuli are visible (Devi et al. 1990, Buckmeier et al. 2002, Khan et al. 2011). However, in other instances lapilli otoliths were sectioned using a saw to attain age estimates (Li and Xie 2008, Hauser et al. 2018). Using the otolith preparation methods described by Buckmeier et al. (2002) allows the otolith to be manipulated while grinding at any angle to expose the nucleus and all annuli. If otolith preparation influences aging precision more than the results found in this study, further research should be conducted to compare age

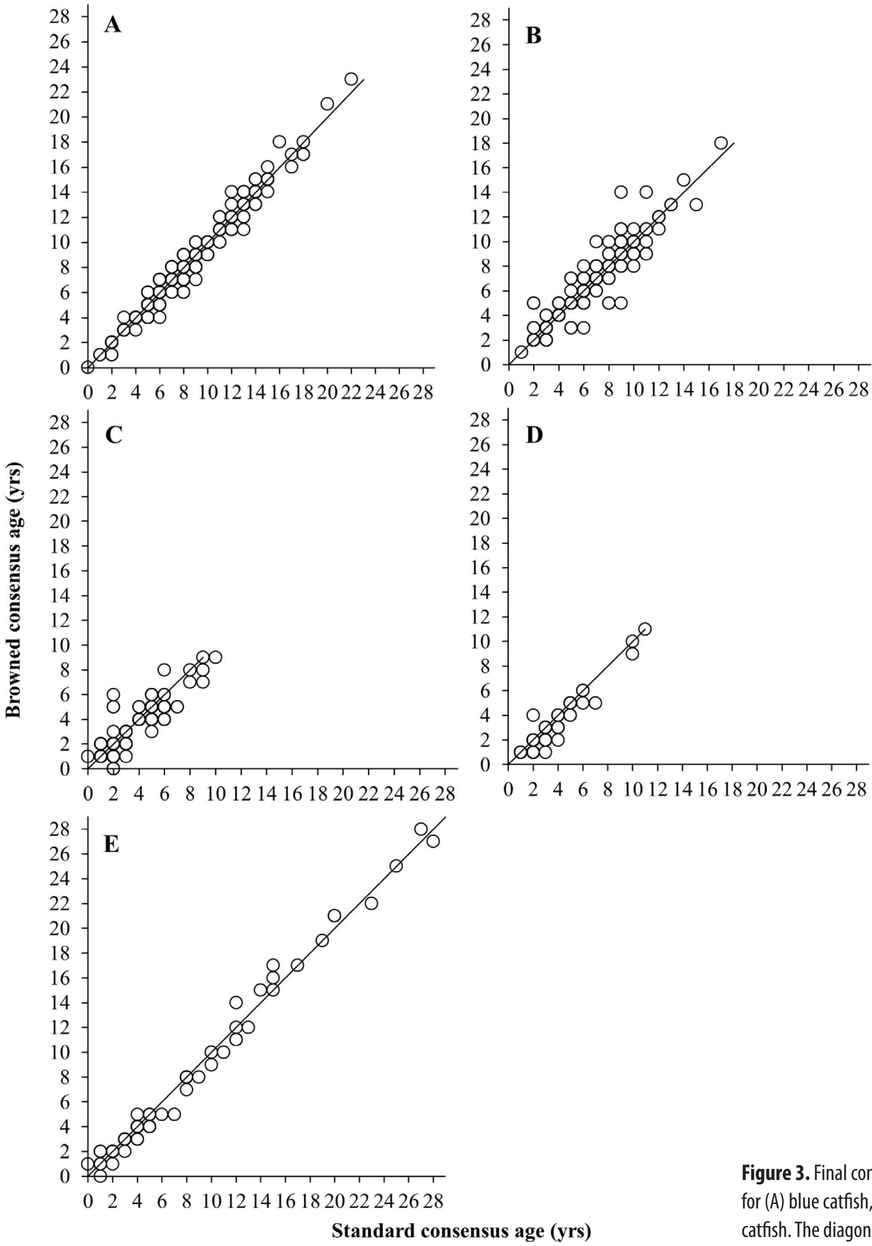

Figure 3. Final consensus age estimates (years) of standard otoliths compared to browned otoliths for (A) blue catfish, (B) channel catfish, (C) black bullhead, (D) yellow bullhead, and (E) flathead catfish. The diagonal line represents a 1:1 relationship between techniques.

estimates from a transverse section with the grinding techniques described by Buckmeier et al. (2002) for all catfish species. Verifying the location of the nucleus in catfish otoliths is critical, as this determines where the otolith needs to be sectioned for age estimation. Biologists should be aware that nucleus location in otoliths can vary considerably among species, even within the same family, and should ensure that this location is properly identified in each study species prior to otolith preparation.

Many studies have browned otoliths to help distinguish annuli, but we did not find that browning otoliths increased precision for estimating age in catfish with the exception of yellow bullhead. Standard otolith preparation time was almost five times faster than when additional browning occurred. Furthermore, readers interpreted otoliths ages without bias compared to consensus ages for each otolith method. The differences observed looking at consensus age bias plots were likely caused during the processing of the otoliths. Our results show that fisheries managers can forego the browning process in most cases and still achieve similar age estimates, saving significant time and monetary cost.

We note that in this study we only address the problem of precision of estimates between methods and not the accuracy of using otolith aging in general, as these were not known-aged fish. We

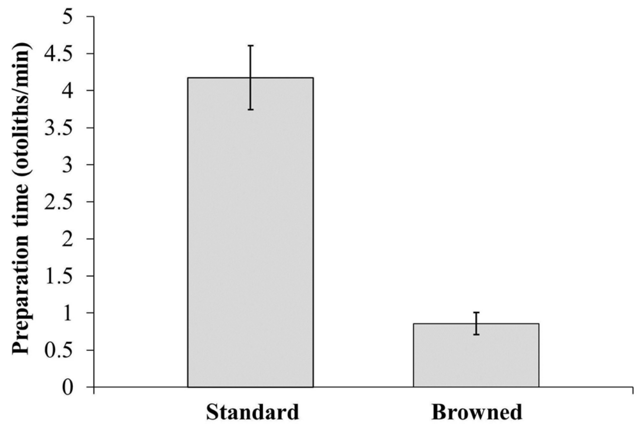

Figure 4. Preparation time (otoliths/min) for both otolith preparation methods (standard and browned). Error bars represent the standard error of the mean.

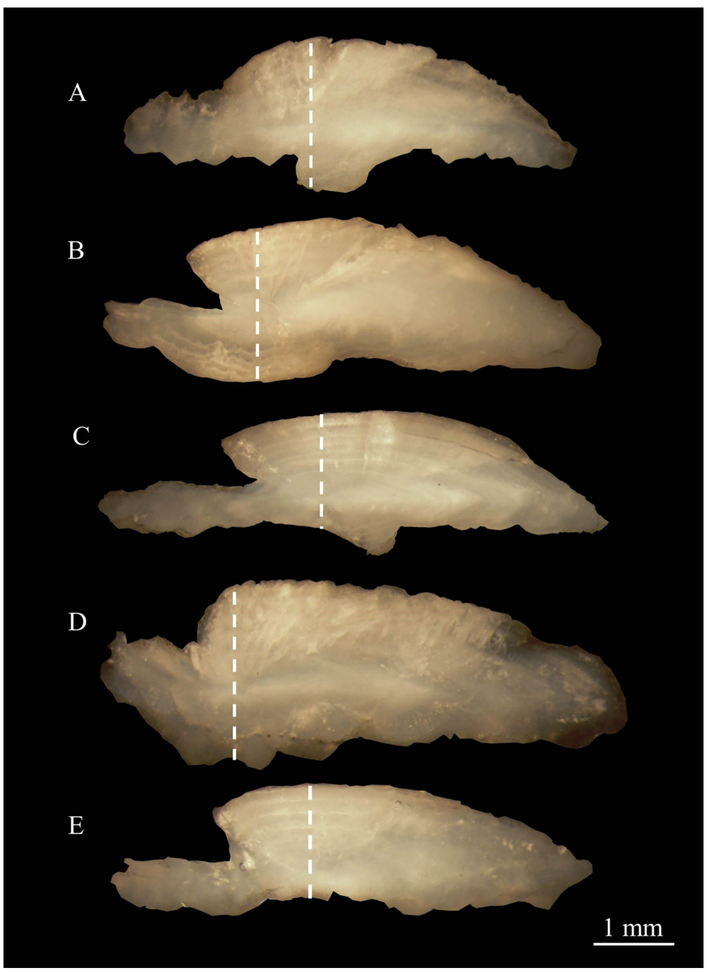

Figure 5. Photograph depicting lapilli otoliths sectioned in a frontal plane of a (A) blue catfish (428 mm TL), (B) channel catfish (380 mm TL), (C) black bullhead (272 mm TL), (D) yellow bullhead (334 mm TL), and (E) flathead catfish. The left side of the images represents the posterior side of the otolith and the right side of the image represents the anterior side of the otolith. The vertical dashed lines show the appropriate transverse sectioning location on each otolith, which is just before the nucleus.

recommend addressing issues of catfish otolith readability by locating the nucleus in the otolith and using the techniques required to best expose annuli based on otolith morphology which would likely improve aging precision for many catfish species.

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