Comparing Precision of Otolith and Pectoral Spine Age Assessments for Black and Yellow Bullheads

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Abstract: Despite the broad geographical range of bullhead catfishes (*Ameiurus* spp.), their population vital rates have rarely been studied. Estimation of vital rates requires accurate age estimates and otoliths generally are considered to be the most accurate and precise aging structure for most fish species. However, pectoral spines of some ictalurid species have been used to generate precise age estimates of younger fish. Although previous studies have compared age estimates between otoliths and spines for large-bodied, longer-lived catfishes, there have been few comparable studies for bullheads. Our objectives were to compare the reader precision and preparation times of lapilli otoliths and pectoral spines (articular process [AP] and basal recess [BR] sections) to determine which aging structure is most precise and efficient for age analysis of black bullhead (*Ameiurus melas*) and yellow bullhead (*A. natalis*). During 2020 we collected 116 black bullhead from River Chase Pond and 106 yellow bullhead from Prague Lake, Oklahoma. Between-reader precision and agreement were higher for lapilli otoliths than the AP and BR sections in both species. As age estimates increased, the AP and BR underestimated ages compared to otoliths in both species. Estimates of von Bertalanffy growth parameters and mortality derived from pectoral spine sections and otoliths differed among structures, with faster growth and higher mortality estimated by spines relative to otoliths. The BR section took the least amount of time to prepare compared to the AP and otoliths but was the least precise. These results suggest that managers should use lapilli otoliths to estimate ages of black bullhead and yellow bullhead.

Key words: catfish, growth, mortality, management, processing time

Journal of the Southeastern Association of Fish and Wildlife Agencies 10:1–9

Reliable age and growth estimates are essential components of effective fisheries management. Together, they contribute to sound management practices and allow estimation of important population dynamics and vital rates (e.g., length-at-age, mortality rate, recruitment variability; Guy and Brown 2007). Fish age can be estimated from structures such as spines, vertebrae, scales, and otoliths, each of which requires different processing methods for interpretation (Campana 2001). Managers require a structure that enables accurate (how closely estimated age reflects true age), precise (how reproducible the measurement is), and efficient (in processing time) age estimation for timely and effective fisheries management (Campana 2001, Buckmeier et al. 2017). However, separate structures (e.g., spines and otoliths) from the same individual can return different age estimates that may lead to different estimates of population vital rates (Olive et al. 2011, Hull et al. 2021). Furthermore, interpretability, or readability, of structures can vary, leading to inconsistencies when ages are estimated by different readers (Porta et al. 2017, Hull et al. 2021).

Lapilli otoliths and pectoral spines are commonly used to estimate ages of ictalurids (Nash and Irwin 1999, Buckmeier et al. 2002, Colombo et al. 2010, Barada et al. 2011, Hull et al. 2021, Sakaris and Bonvechio 2021). Otolith removal requires fish to be sacrificed whereas removal of the pectoral spine appears to have minimal negative effects on ictalurids (Michaletz 2005). Pectoral spines might be more desirable to fisheries managers trying to estimate ages of catfish from small populations or trophy fisheries where

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sacrificing large numbers of fish may not be practical (Michaletz 2005), but these spines are less precise than lapilli (Nash and Irwin 1999, Hull et al. 2021). Pectoral spines are processed at the articular process (AP) and basal recess (BR) section; however, the AP is generally considered more accurate for catfish due to frequent underestimation of fish age from the loss of early annuli in the BR section (Turner 1982, Buckmeier et al. 2002). Although lapilli are generally considered to provide the most precise age estimates for ictalurids (Nash and Irwin 1999, Hull et al. 2021), Colombo et al. (2010) found no significant differences between the age estimates from the AP and otoliths of channel catfish (*Ictalurus punctatus*). This suggests a need to understand the variation in age estimates from different structures (e.g., otoliths vs spines) and from spines processed at different locations (e.g., AP vs BR). Processing times of aging structures should also be examined to determine if preparing a particular structure might be more efficient while still providing quality data. However, only a few studies have examined the processing times of ictalurid aging structures (Barada et al. 2011, Hull et al. 2021). If these structures provide similar age estimates, then using the structure with the least amount of processing time would increase aging efficiency.

Bullhead catfishes (*Ameiurus* spp.) are native to North America east of the Rocky Mountains but have been stocked outside of their native range, including the European continent (Cucherousset et al. 2006, Pedicillo et al. 2008, Novomeská and Kováč 2009). Bullheads can comprise most of the fish biomass in a body of water due to their fast growth and wide niche breadth (Brown et al. 1999, Propst et al. 2015, Snow et al. 2017, Sikora et al. 2021, Sikora et al. 2022). Age and growth information on bullhead catfishes is often lacking compared to other ictalurids (Copp et al. 2016, Jaćimović et al. 2017, Montague et al. 2021) and information such as accuracy, precision, and processing times of pectoral spines and otoliths will increase biologists' ability to effectively manage these species. Therefore, our goal was to determine which structure generated the most precise age estimates of black bullhead (*Ameiurus melas*) and yellow bullhead (*A. natalis*), the two most common bullhead species in Oklahoma. The objectives of our study were to 1) compare the precision of age estimates from each structure for black bullhead and yellow bullhead, 2) evaluate whether pectoral spines sectioned at the AP and BR provide similar growth and mortality estimates to otoliths, and 3) compare the processing time for each aging structure.

Methods

Baited hoop nets were set in March 2020 to collect black bullhead from River Chase Pond (Norman, Oklahoma) and set in September 2020 to collect yellow bullhead from Prague Lake (Prague, Oklahoma). Each fish was measured (TL, mm) and placed into a 1:1 ice water slurry to be euthanized (Blessing et al. 2010). The left pectoral spine was disarticulated (Sneed 1951, Mayhew 1969), removed, boiled to remove organic material (Puchala et al. 2018), placed into individually numbered envelopes, and allowed to dry for at least 168 h before processing (Weber and Brown 2011, Yates et al. 2016). Lapilli otoliths were extracted by cutting with a hacksaw through the supraoccipital bone 3–4 mm anterior to where the pectoral spines are positioned (Buckmeier et al. 2002, Long and Stewart 2010), removed with forceps, cleaned, and placed into individually numbered envelopes and allowed to dry for at least 24 h prior to processing (Secor et al. 1992).

All structures were cut using a low-speed IsoMet saw equipped with a 127-mm diameter × 0.4-mm thickness blade (Model 11-1280-160; Buehler Ltd., Lake Bluff, Illinois) and then polished with wet 2000-grit sandpaper until annuli became clear and distinguishable. Otoliths were prepared for mounting by positioning the otolith distal side facing up in individual cells of a silicon mold (Electron Microscopy Sciences, Fort Washington, Pennsylvania) and covered with epoxy (West System 205-B hardener and 105-B Epoxy resin, Gougeon Brothers Inc., Bay City, Michigan; Sakaris et al. 2017, Waters et al. 2019). The AP was processed by cutting with the IsoMet saw at the AP portion of the spine (Buckmeier et al. 2002). The BR region of the pectoral spines was processed by cutting just anterior to the BR and polishing after ages were estimated from the AP section (Murie et al. 2009). Otoliths were submerged in water within a dish containing black modeling clay and the polished side up viewed using a dissecting microscope (4×–90× magnification). The AP and BR sections of the pectoral spine were viewed by placing the distal end into clay with the polished side facing up, leveled, and coated with mineral oil to improve clarity. All annular marks were illuminated using a fiber optic filament attached to an external light source (Porta et al. 2017). When illuminated, annular marks appeared as opaque bands on a lighted background for otoliths and as translucent bands on a light background for spines (Snow et al. 2018).

Two readers independently estimated fish age using all structures for both species by evaluating each structure separately in random order (Hoff et al. 1997). Each reader had ample aging experience (reader 1, 13 years; reader 2, 5 years) with freshwater fish using various structures. If independent age estimates disagreed between readers, a concert read was conducted by both readers simultaneously to reach an agreement. If an agreement could not be reached between readers, that structure was deemed unreadable and removed from the study; none of the structures from that fish were compared with a final consensus reading (Hoff et al. 1997).

Preparation time (min) for each structure (AP, BR, and otoliths) was recorded as the time required to clean, section, and polish prior to age estimation. For pectoral spines, this included the time to boil and remove excess organic material; otolith preparation included time to remove from envelopes, orient in silicon molds, and apply epoxy to wells. A single timed trial was conducted by batch sampling a haphazardly chosen subset of 13 to 18 unique structures (i.e., either all pectoral spines or all otoliths), recording the time required to process those structures (min), then dividing the number of structures by the total processing time. This process was replicated seven times for each structure. Preparation time estimates were calculated as the number of structures processed in one minute. Structures were selected without knowledge of the bullhead species they came from as we did not hypothesize that processing time would differ between species.

Data Analyses

Between-reader precision of age estimates was evaluated using percent reader agreement (Campana et al. 1995), average percent error (APE; Beamish and Fournier 1981), CV (Chang 1982), and paired *t*-tests (Hurley et al. 2004), the latter using XLSTAT (Addinsoft Inc., New York City, New York). Paired *t*-tests with XLSTAT were also used to compare the consensus ages between structure pairs from each species. Cohen's d statistic was used to estimate the effect size of all paired *t*-tests, where d values of 0.20, 0.50, and 0.80 were used as the thresholds for small, moderate, and large differences, respectively (Cohen 1988). Age-bias plots were used to qualitatively compare individual age estimates from readers to the final consensus age estimate for each structure and species. Age-bias plots were also used to qualitatively compare final consensus ages between structures for each species.

Growth and instantaneous mortality rates for both species were estimated independently using each structure to determine if estimates of population vital rates varied between structures. Growth was estimated using a von Bertalanffy (1938) growth model fit to TL and age estimates using the Fisheries Stock Analysis R package (Ogle et al. 2021). We then compared parameter estimates (L_∞, k, and t_0) obtained using otoliths to those obtained using pectoral spines sectioned at the AP or BR with a likelihood ratio test (Kimura 1980, Ogle 2016, Porta et al. 2017). Likelihood ratio tests were conducted with the fishmethods R package (Nelson 2019). Instantaneous mortality rates (Z) were estimated via a weighted catch curve fit to estimated ages using the Fisheries Stock Analysis R package (Ogle et al. 2021). Total annual mortality (A) was also estimated for each structure using $1\text{-}e^{-z}$ (Ricker 1975). Slopes of catch curves obtained from otoliths were compared to those from pectoral spines sectioned at the AP or BR using an ANCOVA in XLSTAT. A one-way ANOVA with a post hoc Tukey's honestly significant difference test was conducted in program R version 4.1.2 (R Core Team 2021) to determine if the processing times differed significantly between structures. The assumptions of normality for ANOVA were confirmed via residual diagnostic plots. All statistical results were considered significant at $P < 0.05$.

Results

Age Estimation

Black bullheads ($n = 116$) ranged 67–404 mm TL and yellow bullheads ($n = 106$) 145–339 mm TL. Structures used for comparison in our analyses were obtained from 114 of 116 black bullheads and all 106 yellow bullhead. Black bullhead age estimates ranged from 1 to 12 years for otoliths, 1 to 7 years for AP sectioned spines, and 1 to 8 years for BR sectioned spines. Yellow bullhead age estimates ranged from 1 to 6 years for otoliths and 1 to 5 years for both spine sections.

Between-reader precision yielded mixed results for structures and species. Age-bias plots suggested that BR was the least precise structure for both species, whereas otoliths and AP had similar precision (Figure 1). Summary statistics (Table 1) suggested otoliths were the most precise structure for both species, with AP intermediate and BR least precise. Age estimates between readers were similar for yellow bullhead when using otoliths and for both species when using the BR, with effect-size estimates for these comparisons below the threshold for even a small effect. Interestingly, age estimates between readers were different for black bullhead when using otoliths and for both species when using the AP, although corresponding effect-size estimates suggested that differences were small.

Age-bias plots comparing consensus ages obtained from each structure yielded similar results for both species. The AP showed higher precision for younger fish of both species. However, the AP began to underestimate age compared to otoliths as age increased above age 5 for black bullhead and above age 4 for yellow bullhead

Table 1. Number of samples (*n*), percent reader agreement, average percent error (APE), and mean CV (%) for ages estimated between lapilli otoliths, spines sectioned at the articular processes, and spines sectioned at the basal recess for black bullhead and yellow bullhead. Included are results from paired *t*-tests and effect-size estimates (Cohen's d) comparing initial age estimates from two readers for each structure and species.

Species	Structure	*n*	% Agreement	APE	Mean CV	*t*	df	*P*	Cohen's d
Black bullhead	Otolith	115	91.3	0.7	1.1	2.92	114	0.04	0.27
	Articular process	114	71.7	4.2	5.9	2.10	113	0.02	0.20
	Basal recess	116	69.8	5.1	7.3	0.93	115	0.17	0.09
Yellow bullhead	Otolith	106	95.3	0.7	1.0	0.44	105	0.66	0.04
	Articular process	106	80.2	5.1	7.3	3.12	105	0.01	0.30
	Basal recess	106	69.8	7.4	10.4	0	105	1	<0.01

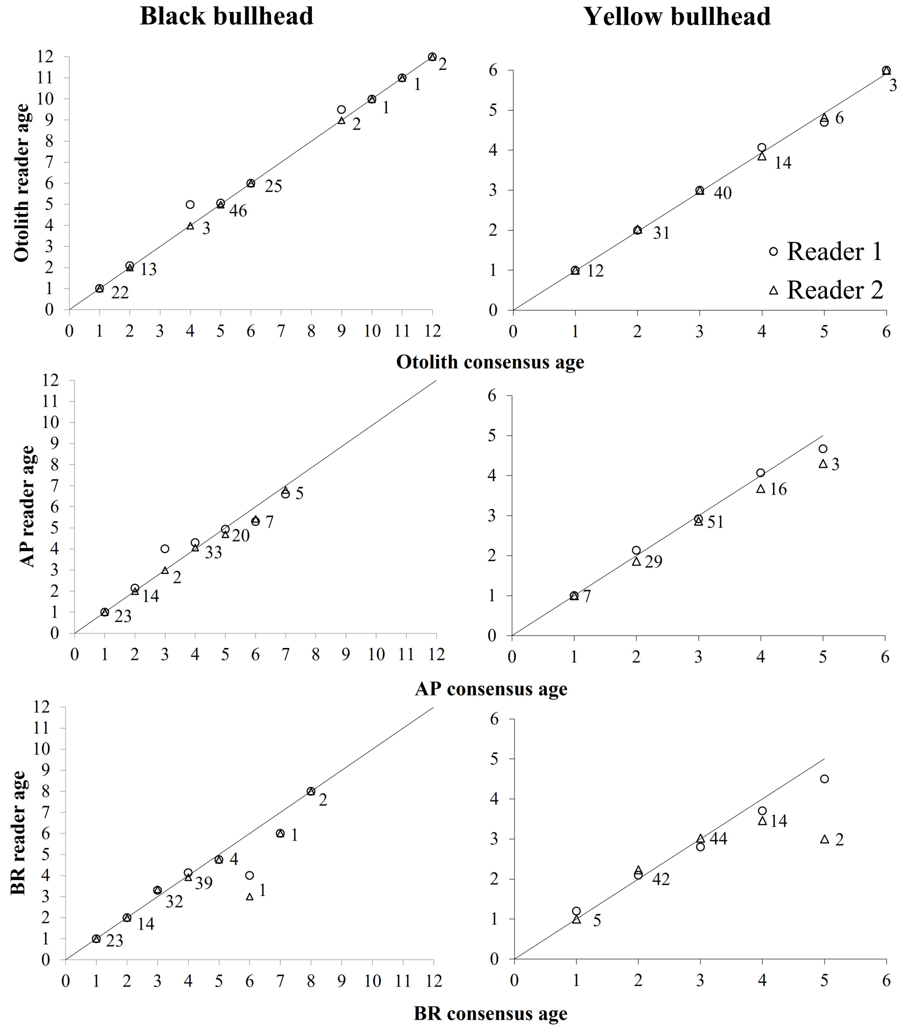

Figure 1. Age-bias plots comparing reader 1 and reader 2 age estimates (years) from otoliths, articular processes (AP), and basal recesses (BR) of black bullhead and yellow bullhead to final consensus age estimates for each structure. Error bars represent 95% CI. The diagonal line represents 100% agreement between consensus and reader 1 and 2 age estimates. Numbers adjacent to each point indicate sample size of each age group.

(Figure 1). The BR produced low precision for both species, particularly for older fish. For both species, age-bias plots showed higher precision between readers when estimating age from otoliths compared to the final consensus estimated age. Spines (both AP and BR) overestimated ages compared to otoliths in younger yellow bullheads and underestimated ages compared to otoliths starting at age 4 for both species (Figure 2). The BR underestimated ages starting at age 5 for black bullhead and age 4 for yellow bullhead compared to the AP.

Comparisons of consensus ages from each structure yielded different patterns for the two species. Age estimates from black bullhead structures were all different from each other (Table 2). Effect-size estimates suggested moderate differences between age estimates from AP and otoliths and between age estimates from AP and BR. Cohen's d suggested there were strong differences between age estimates from BR and otoliths (Table 2). Conversely, age estimates of yellow bullhead were similar for all structures and our effect-size estimates confirmed this with all d values falling below the threshold for a small effect.

Table 2. Comparison of ages estimated between lapilli otoliths (Oto), spines sectioned at the articular processes (AP), and spines sectioned at the basal recess (BR) for black bullhead and yellow bullhead. Included are paired *t*-test results and effect-size estimates (Cohen's d) comparing consensus age estimates from two readers for each structure and species.

Species	Comparison	*t*	df	*P*	Cohen's d
Black bullhead	Oto vs. AP	6.01	112	0.01	0.57
	Oto vs. BR	9.43	114	0.01	0.88
	AP vs. BR	6.18	113	0.01	0.58
Yellow bullhead	Oto vs. AP	0.11	105	0.91	<0.01
	Oto vs. BR	1.62	105	0.11	0.18
	AP vs. BR	1.56	105	0.12	0.15

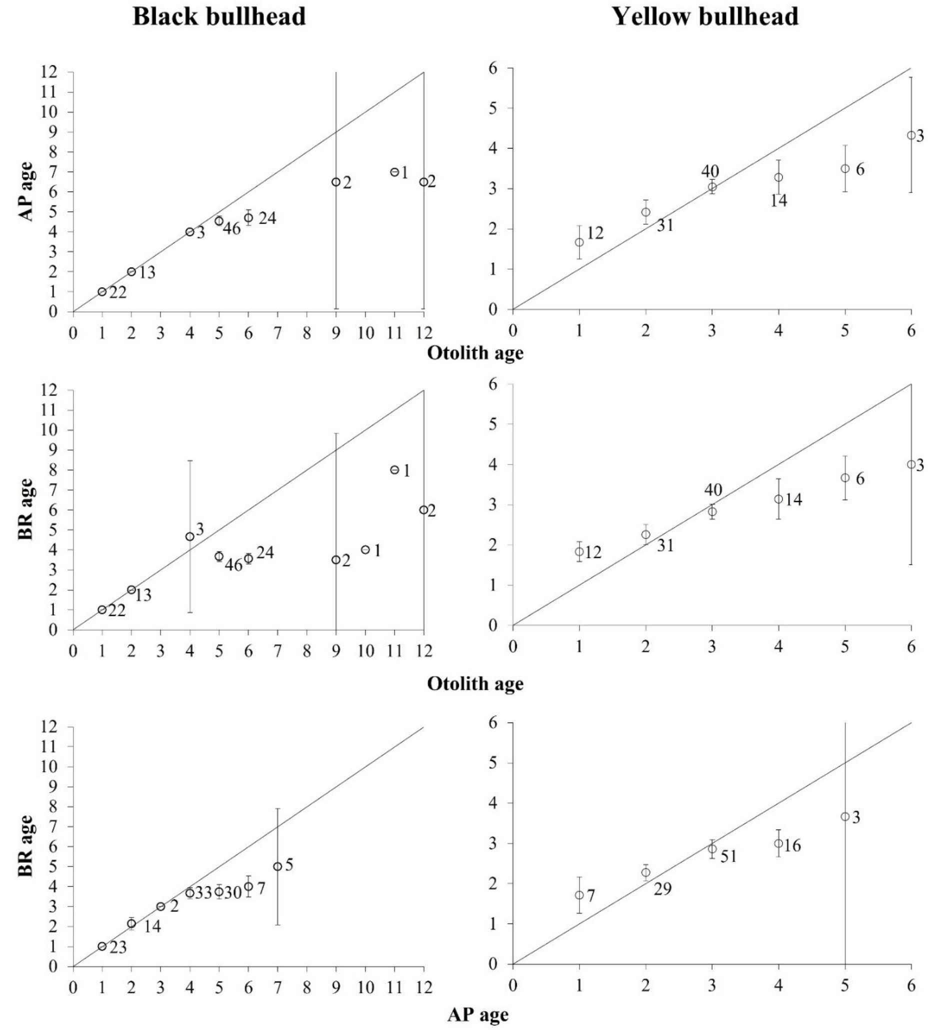

Figure 2. Age-bias plots comparing final consensus age estimates (years) of otoliths to articular processes (AP) and basal recesses (BR) of black bullhead and yellow bullhead. Error bars represent 95% CI. The diagonal line represents 100% agreement between structures. Numbers adjacent to each point indicate sample size of each age group.

Table 3. Growth parameters from von Bertalanffy growth models, results from likelihood ratio tests (LRT) used to compare growth parameters, instantaneous total mortality (*Z*), total annual mortality (A), and ANCOVA results comparing slopes of catch curves (*Z*) calculated using age estimates from otoliths, spines sectioned at the articular processes, and spines sectioned at the basal recess for black and yellow bullhead from two Oklahoma reservoirs. The ANCOVA results compare slopes from otolith vs. articular process and basal recess estimates.

		Growth parameters			LRT[a]		Mortality		ANCOVA	
Species	**Structure**	**L∞**	**k**	**t_0**	**X^2**	***P***	***Z***	**A**	***F***	***P***
Black bullhead	Otolith	470	0.10	−1.33			−0.48	0.38		
	Articular process	280	0.45	0.26	3.14	0.37	−0.71	0.51	2.44	0.17
	Basal recess	299	0.34	0.45	3.25	0.36	−0.74	0.52	8.93	0.02
Yellow bullhead	Otolith	407	0.23	−1.66			−0.86	0.55		
	Articular process	314	0.41	−1.33	11.15	0.01	−1.42	0.78	0.57	0.49
	Basal recess	382	0.27	−1.80	4.13	0.25	−1.56	0.79	1.89	0.24

a. df = 3 for each LRT.

Vital rates derived from otoliths and spine sections exhibited a similar pattern regardless of species. Mean parameter estimates from growth curves fit using otolith age estimates predicted that both species would reach larger sizes (L_∞) and grow slower (k) compared to growth curves fit using AP or BR age estimates (Table 3). However, likelihood ratio tests suggested growth parameter estimates were similar among structures for black bullhead and between BR and otoliths for yellow bullheads. Age estimates from otoliths for both species resulted in the lowest estimate of instantaneous mortality (Z) and, by extension, total annual mortality (A; Table 3). However, ANCOVA results suggested that Z was only different between BR and otoliths for black bullheads.

Preparation Time

A total of 106 otoliths, 104 AP sections, and 106 BR sections were used during our processing trials. Processing rate was fastest

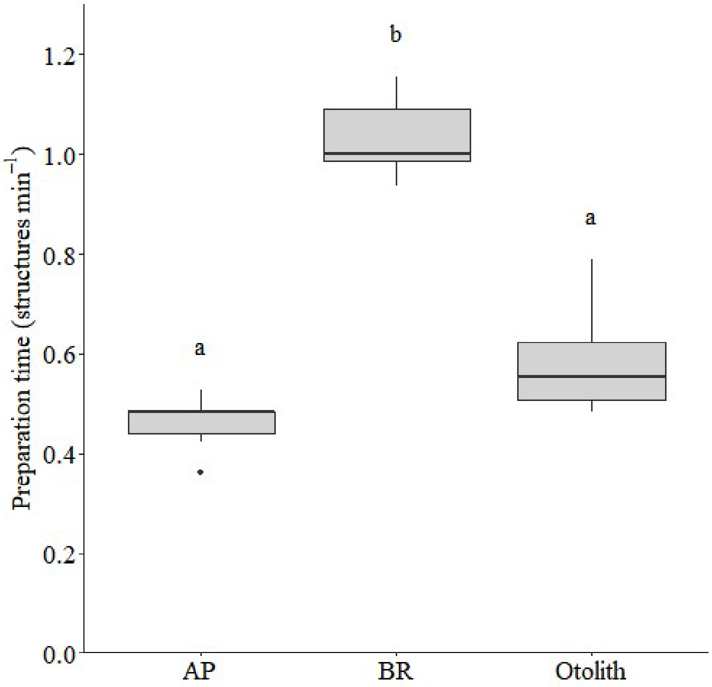

Figure 3. Boxplots displaying the distribution of observed preparation times (structures min^{-1}) for each structure from bullheads. Bold line represents mean preparation time; the box represents the 25th and 75th percentiles; whiskers represent likely maximum and minimum values (excluding outliers); and dots represent suspected outliers. Letters above boxes represent statistically different groups based on an ANOVA with a Tukey's honestly significant difference post hoc test.

for BR ($\bar{x} = 1.03$ structures min^{-1}, range = 0.94–1.15), followed by otoliths ($\bar{x} = 0.58$ min^{-1}, range = 0.48–0.79), and AP ($\bar{x} = 0.46$ min^{-1}, range = 0.36–0.53), and was different among structures ($F_{2,18} = 89$, $P < 0.01$). Tukey's honestly significant difference post hoc test suggested that processing rate was significantly faster for BR than AP and otoliths ($P < 0.01$; Figure 3).

Discussion

We found that for both bullhead species, lapilli otoliths generated more precise age estimates compared to pectoral spines (both the AP and BR sections). These results were similar to other studies that found high (>90%) percent agreement for otoliths (Maceina and Sammons 2006, Montague et al. 2021). Precision of otoliths from these two bullhead species was greater than those reported for other ictalurids including brown bullhead (*Ameiurus nebulosus*; Maciena and Sammons 2006), channel catfish (Buckmeier et al. 2002, Barada et al. 2011, Hull et al. 2021), flathead catfish (*Pylodictis olivaris*; Nash and Irwin 1999), and blue catfish (*Ictalurus furcatus*; Olive et al. 2011). In addition, our results agree with previous studies indicating that the AP was more precise than the BR (Crumpton et al. 1984, Buckmeier et al. 2002). Regardless, spines sectioned at either location began to underestimate ages relative to the otolith after age 3, suggesting they are of limited utility for estimating bullhead ages. Pectoral spines sectioned at the BR likely underestimated ages of bullhead due to the expansion of the central lumen (Nash and Irwin 1999, Buckmeier et al. 2002, Hull et al. 2021). Although it is unclear why sections from the AP also underestimated age, other studies have also found that AP sections underestimate ages of catfish (Nash and Irwin 1999, Buckmeier et al. 2002, Maceina and Sammons 2006). Our findings indicate that lapilli otoliths age estimates were highly precise up to age 12 for black bullhead and age 6 for yellow bullhead.

Our finding that otoliths are the more precise aging structures than other, non-lethal structures for age estimation is consistent with previous age comparison studies conducted on various fish families. For percids, Porta et al. (2017) found that dorsal spines overestimated ages compared to otoliths in saugeye (*Sander vitreus* × *S. canadensis*) and Dembkowski et al. (2017) found that walleye (*Sander vitreus*) dorsal spine ages underestimated ages after 7 years compared to otoliths. Estimates from spines, fin rays, and scales frequently underestimated ages compared to otoliths for alligator gar (*Atractosteus spatula*; Buckmeier et al. 2012). Estimates derived from branchiostegal rays and sectioned pectoral fin rays also underestimated ages of longnose gar (*Lepisosteus osseus*) and spotted gar (*Lepisosteus oculatus*; Buckmeier et al. 2018).

Parameter estimates from von Bertalanffy growth model using ages estimated from the AP and BR appeared different from otolith-derived ages, but parameter estimates only differed between yellow bullhead AP and otoliths. This was surprising because the disparity in growth curve mean parameter estimates obtained from black bullhead otoliths and both spine sections appeared greater than yellow bullhead otoliths and AP sections. High individual variability in growth trajectories for black bullhead likely limited our ability to detect a change. These discrepancies may have reflected differences in populations rather than fixed differences between species, as Hull et al. (2021) found growth parameter estimates derived from ages estimated from pectoral spines and otoliths differed in only one of two channel catfish populations studied. Variation in growth trajectories within and between years in populations and its potential influence on management decisions is poorly understood for fishes and should be a topic of further study.

Important differences, such as in maximum age and missing year classes, were observed among age estimates of otoliths and spines in our study. For example, black bullhead maximum age was estimated to be 12 for otoliths but only 7 and 8 when using spines sectioned at the AP and BR, respectively. Conversely, the yellow bullhead population was younger, and we observed less of a difference between maximum ages of otoliths (maximum age = 6) and spines (maximum age = 5 for both AP and BR). As longevity of fish species increases, differences in age estimates among structures usually show a commensurate increase (Maceina and Sammons 2006), consistent with what we observed in our study. Furthermore, the missing year classes observed with black bullhead otoliths could depict years where recruitment was poor and would have gone unobserved if only pectoral spines (AP or BR) were used. Data from pectoral spines lacked missing year classes, suggesting that the population has had consistent recruitment, whereas otoliths suggested the population has had variable recruitment. The stable recruitment suggested by pectoral spines for black bullhead in River Chase Pond is unlikely as previous research has documented variable recruitment for a variety of ictalurids (e.g., Hubert 1999, Holley 2006, Mork et al. 2009, Kuklinski and Patterson 2011, Settineri 2015, Duck 2020). Thus, choice of aging structure can affect a variety of population metrics and potentially misinform biologists if the wrong structure is used.

Our finding that otoliths and spines sectioned at the AP had similar processing times is likely the result of not incorporating removal time into our preparation time calculation. Although the BR took less time to prepare, sections from pectoral spines were less precise than otoliths. Therefore, we recommend managers take the additional time to process otoliths to obtain the most precise age estimates. Our study did not examine reading and removal times or the experience of structure processor (i.e., some professionals may be more efficient than others); future research should consider the entirety of the age estimating process (removal, preparing, and reading of the structures) while noting the experience of the processor.

Our results support the use of lapilli otoliths as precise and efficient aging structures and increase our understanding of age estimates for both bullhead species. This information is useful to biologists managing bullheads and aids in understanding bullhead life history. However, neither this study nor any previous studies have examined the accuracy of either bullhead species' aging structures. Otolith validation has been conducted for other fish species, including catfish species, suggesting that otoliths are the structure most likely to provide accurate estimates (Buckmeier and Smith 2020, Walker et al. 2020, Chhuoy et al. 2022). Otolith validation needs to be examined for bullhead catfishes to confirm that otoliths provide accurate age information, which is crucially needed for understanding bullhead life history, recruitment, and population characteristics.

Acknowledgments

We thank Jory Bartnicki, Shelby Jeter, Michael Porta, Keith Thomas, and Michael Holley for assisting in field sampling and lab work for this project. We thank Dr. James Long (Oklahoma State University) for reviewing an earlier draft of this manuscript. Financial support for this publication was provided by the Sport Fish Restoration Program grants [F-50-R-25] and [F-86-D-1] to the Oklahoma Department of Wildlife Conservation.

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