Bias, Precision, and Processing Time of Otoliths and Pectoral Spines Used for Age Estimation of Channel Catfish

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Abstract.—Otoliths and pectoral spines are common calcified structures used to age channel catfish *Ictalurus punctatus*. Several studies have assessed accuracy and precision of these structures; however, results have been conflicting. Additionally, information on processing times required to prepare and assign ages to these structures is lacking. Therefore, our objective was to assess bias, precision, and processing time of otoliths and pectoral spines used for age estimation of channel catfish. Otoliths (N = 603) and spines (N = 3,397) were collected from channel catfish during 2007–2009 to assess bias and precision. A subsample of these structures was used to estimate and compare processing times. Otoliths displayed greater precision and less reader bias compared to pectoral spines. Age-specific bias was observed for pectoral spines in relation to otoliths, with pectoral spines greatly underestimating ages for age-11 and older channel catfish. Total processing time was greater for otoliths (12.26 min ± 1.09 [SE]) compared to pectoral spines (11.01 min ± 0.07 [SE]). These results provide managers with information to make decisions on which structure to use for age estimation of channel catfish given a known bias and precision desired for statistical analyses and amount of time allotted for the aging process.

Introduction

Age data are often used to estimate growth characteristics, age distribution, and mortality of fish populations (Devries and Frie 1996). Therefore, accurate age estimates are essential when assessing fish population dynamics to guide resource management. Numerous hard parts have historically been used to estimate catfish genera ages, including vertebrae (Appelget and Smith 1951; Marzolf 1955), dorsal fin spines (Layher 1981), pectoral fin spines (Mayhew 1969; Prentice and Whiteside 1975; Holland and Peters 1992; Shephard and Jackson 2006), and otoliths (Crumpton et al. 1987; Nash and Irwin 1999; Buckmeier et al. 2002). Pectoral fin spines and otoliths are most commonly utilized today (Maceina et al. 2007). Otolith removal requires fish to be killed. However, pectoral spine removal causes little if any mortality (Stevenson and Day 1987; Michaletz 2005) and is preferred when sacrificing fish is undesirable.

Evaluating different age estimation techniques is important to ensure accuracy, precision, and time efficiency. However, few channel catfish *Ictalurus punctatus* age structure assessment investigations have been conducted, and results from these studies are conflicting. Prentice and Whiteside (1975) validated basal recess (BR) pectoral spine cross sections for age-1 to age-4 channel catfish, observing no trends in under- or over-aging of known-age fish. Crumpton et al. (1987) compared age estimates from otoliths to BR, midspine, and articulating process (AP) pectoral spine cross sections. Results indicated identical age estimates among all spine sections (age 2 to age 7). However, otoliths were deemed unacceptable for channel catfish age estimation. Buckmeier et al. (2002) used known age (age 1 to age 4) channel catfish to measure accuracy and validate age estimations for pectoral spines and otoliths. They found pectoral spine (AP sections and a new "cut spine" method) age estimates were more variable and tended to overestimate fish ages compared to otoliths. Ultimately, otoliths were recommended for channel catfish age estimation because of their accuracy and low variability (Buckmeier et al. 2002). More recently, Michaletz et al. (2009) evaluated accuracy and precision of back-calculations from channel catfish structures and deemed otoliths not useful for back-calculation.

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Valuable information has been gained from these channel catfish structure comparisons. However, contrasting results from individual studies have complicated overall conclusions, and no studies have assessed processing time for complete analysis. Additionally, the above studies have been conducted in southern latitudes of the channel catfish geographic range, using young fish (age 1 to age 7) collected from lentic habitats. Addressing issues concerning aging structures is beneficial to fisheries managers aiming to efficiently collect sound population dynamics information. Therefore, our objective was to evaluate common hard structures used for channel catfish age estimation, specifically estimating bias, precision, and processing time of otoliths and pectoral spines from channel catfish.

Methods

Field Collections

Channel catfish were collected using hoop nets, electrofishing, and trotlines in the Platte River, Nebraska (river kilometer 1–370). Fish sampling was conducted during spring, summer, and fall from 2007 to 2009. Captured catfish were measured for total length (mm) and weight (g). Pectoral spines were removed from a subsample for age and growth analysis. Additional fish were collected from angler tournaments held on the Platte River throughout the study period. Angler caught catfish were also measured for length and weight at the angler check-in, and fish were tagged with individually numbered floy tags (Floy Tag Inc., Seattle, Washington) before being cleaned by the anglers. Heads (floy tags intact) of individually numbered filleted catfish were then kept on ice and transported back to the laboratory for spine and otolith removal.

Laboratory Preparation

Pectoral spines were prepared using modified methods from Koch and Quist (2007), and otoliths were processed following methods from Buckmeier et al. (2002). Both pectoral spine and otolith processing consisted of removal, setting, cutting or sanding, image capturing, and reading. However, pectoral spine processing consisted of additional procedures that included filling, popping, and cytosealing. Processing times of specific procedures used to prepare and age pectoral spines and otoliths were recorded for a subsample of fish (N = 286) to evaluate processing time differences.

Pectoral spine processing.—Pectoral spine removal consisted of extracting the spine from a channel catfish, transferring the structure to a coin envelope, and recording specific information for the structure (date, unique identifier, fish number, length, weight, and species code) on each envelope. Spine setting consisted of cleaning the spine of excess tissue, cutting the ends of 2.0-mL (for spines of fish < 300 mm) or 5.0-mL (for spines of fish ≥ 300 mm) flat-top microcentrifuge tubes (Fisher Scientific, Pittsburgh, Pennsylvania), filling the inner portion of the tube caps with modeling clay, setting the end of the spine into the clay filled cap, and numbering each tube with unique information. Tube filling consisted of filling each tube with epoxy (Epoxicure Epoxy Resin and Hardener, Buehler Inc., Lake Bluff, Illinois) to encase the entire spine. Spines were embedded in epoxy to reduce damage and facilitate manipulation during cutting. Spine popping consisted of removing or "popping" each spine from the centrifuge tube after hardening and placing the encapsulated spine back into the original envelope. Spine cutting consisted of securing the spine in a low-speed saw (Isomet 1000, Buehler Inc., Lake Bluff, Illinois), cutting three cross sections, 0.7 mm thick, at the distal end of the basal process of each spine (Sneed 1951; Crumpton et al. 1987) and placing spine sections back into the envelope. Cytosealing consisted of placing cross sections on a glass microscope slide, dripping Cytoseal mounting medium (Richard-Allan Scientific, Kalamazoo, Michigan) over each of the cross sections, and transferring unique fish information to the microscope slide. Image capturing consisted of placing mounted spine cross sections under a microscope (Olympus SZ61, Olympus, Center Valley, Pennsylvania), observing all cross sections to pinpoint the one that displayed the most discernible annuli, and capturing the image of that cross section using a high-resolution digital camera (Olympus DP20, Olympus, Center Valley, Pennsylvania) mounted on the microscope. Reading consisted of retrieving the picture file on the computer, counting annuli, and transferring determined age to a spreadsheet.

Otolith processing.—Otolith removal consisted of extracting the otolith from a channel catfish, transferring the structure to a coin envelope, and recording the specific information for the structure on each envelope. Otolith setting consisted of browning each otolith on a hot plate to increase readability of annuli, mounting on a microscope slide using Crystalbond adhesive (Buehler Inc., Lake Bluff, Illinois) with the rounded anterior edge upright and the pointed posterior edge in contact with the slide, then transferring the specific information to the slide. Otolith sanding consisted of sanding one-third to one-half of the otolith with wet 400 grit sand paper to reveal the nucleus. Image capturing consisted of polishing the otolith with wet 600 grit sand paper, examining the otolith under a microscope using side illumination (reflecting light at several angles) until annuli were clearly revealed, and capturing the image using a high-resolution digital camera mounted on the microscope. Reading consisted of retrieving the picture file on the computer, counting annuli, and transferring determined age to a spreadsheet. All structures were aged independently by the same two readers throughout the study. Disagreements in age estimates were resolved with a concert reading.

Data Analyses

Between-reader (Reader 1 versus Reader 2) and between-structure (pectoral spine versus otolith) precision and bias were analyzed. Individual reader ages were used for between-reader analysis, whereas reader consensus ages were used for between-structure comparisons. Presence of bias confounds interpretation of most measures of precision (Campana et al. 1995). Therefore, analyses initially addressed possibility of systematic differences. Scatter plots were first constructed and simple linear regression analysis was used to identify relative under- and/or overestimation. Visual observations of these plots do not always typify amount of bias present because coincident points are not shown. Additionally, age-estimation bias does not always follow a linear pattern. Therefore, we also constructed age-bias plots with 95% confidence intervals to further analyze systematic differences. Age-bias plots are more sensitive to nonlinear bias across all age-classes (Campana et al. 1995). Age-bias plots depict mean age assigned by one reader for all fish assigned a given age by a second reader (Stolarski and Hartman 2008). Therefore, specific age-class bias can be detected by visual observations of plot deviance from a 1:1 equivalence line.

Percent exact agreement (PA-0), percent agreement within 1 year (PA-1), and percent agreement within 2 years (PA-2) were used to measure precision for between-reader and between-structure comparisons. All otoliths were aged over the same time period (2009). However, pectoral spines were aged after each year of collection (i.e., 2007, 2008, and 2009). Readers had some experience aging fish using different hard parts. However, neither reader had experience aging channel catfish using pectoral spines at the beginning of the study. Therefore, we also analyzed pectoral spine precision and bias in terms of reader experience by year.

Mean processing times were calculated for individual methods used to prepare and age spines and otoliths. Individual method times were then summed for a total processing time for each structure. Standard errors and variances were also calculated for each method, and *t*-tests were conducted (SAS Institute 2004) to test for differences ($\alpha = 0.05$) in mean processing times.

Results

A total of 4,000 channel catfish age structures was collected and aged during 2007 to 2009. The majority (85%) of structures used for age estimations were pectoral spines (N = 3,397). However, 603 otoliths were obtained from tournament angler catches (Table 1). Paired samples of pectoral spines and otoliths were collected from 525 fish for age structure comparisons. Estimated ages ranged from 0 to 16 years.

TABLE 1. Between-reader and between-structure percent exact agreement (PA-0), percent agreement within 1 year (PA-1), and percent agreement within 2 years (PA-2) in age estimates assigned by two readers for pectoral spines and otoliths of channel catfish from the Platte River, Nebraska.

	Agreement between readers					Agreement between structures
	Pectoral spines				Otoliths	
	2007	2008	2009	2007–2009	2007–2009	
N	530	1,425	1,442	3,397	603	525
PA-0 (%)	47	67	78	68	78	45
PA-1 (%)	91	97	98	97	97	83
PA-2 (%)	98	99	100	99	99	94

Observations of scatter plot regressions indicate more between-reader bias for pectoral spines compared to otoliths (Figure 1). Otoliths appear to display very little age-estimation bias with a regression slope very close to 1 (b = 0.958; Figure 1). Age-bias plots indicate low between-reader bias for pectoral spines and otoliths at most ages. Noticeable deviations from the 1:1 equivalence line for pectoral spines appear in age-0 and age-11 and older fish, whereas deviations occur mainly in age-11 and older fish for otoliths (Figure 1). Otoliths displayed the greatest precision as percent exact agreement was 78% for otoliths compared to 68% for pectoral spines (Table 1).

Reader experience appeared to affect reader bias and precision. Scatter plot regressions displayed between-reader bias for pectoral spines aged by inexperienced readers (2007; Figure 2). However, little bias was observed for age estimates assigned by the same readers with more experience and familiarity with the aging structure (2008 and 2009; Figure 2). Slopes and r^2 values moved closer to 1.0 with each year of experience. Age-bias plots further indicate between reader bias for 2007 pectoral spines where Reader 2 slightly underestimated age-3 and older fish relative to estimates of Reader 1 (Figure 2). Additional observations of age-bias plots indicate much less reader bias for 2008 and 2009 pectoral spines (Figure 2). Exact percent agreement (P-0) between readers was much lower for pectoral spines aged during 2007 (46%) compared to 2008 (67%) and 2009 (78%).

Bias between pectoral spines and otoliths was observed from scatter plot regressions (Figure 3). Pectoral spines consistently led to younger age estimates relative to otolith age estimates. Observations of age-bias plots reveal between-structure bias for specific age-classes of channel catfish (Figure 3). Pectoral spine and otolith age estimates are similar for age-4 to age-6 channel catfish. However, pectoral spines overestimate age-3 fish and underestimate age-7 and older fish in relation to otolith age estimates. It is important to note that only slight under-

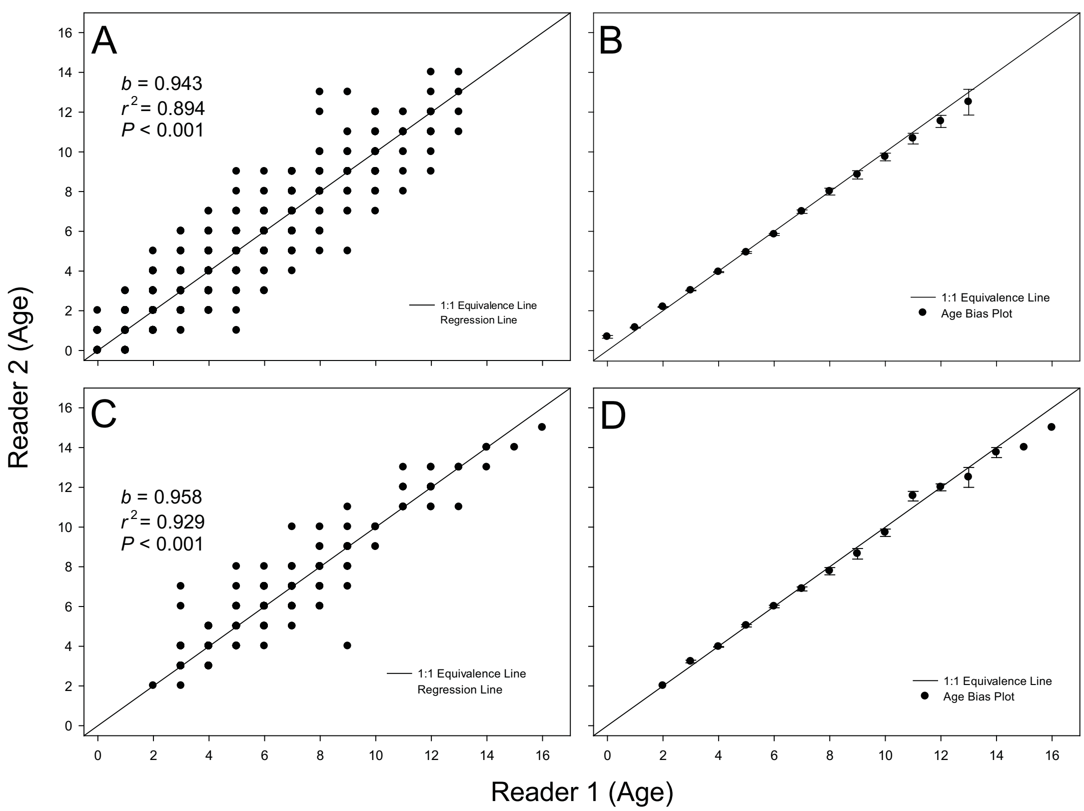

FIGURE 1. Scatter plot regressions and age-bias plots of channel catfish age estimates assigned by two readers for pectoral spines (A, B) and otoliths (C, D) compared to a 1:1 equivalence line (b = regression slope).

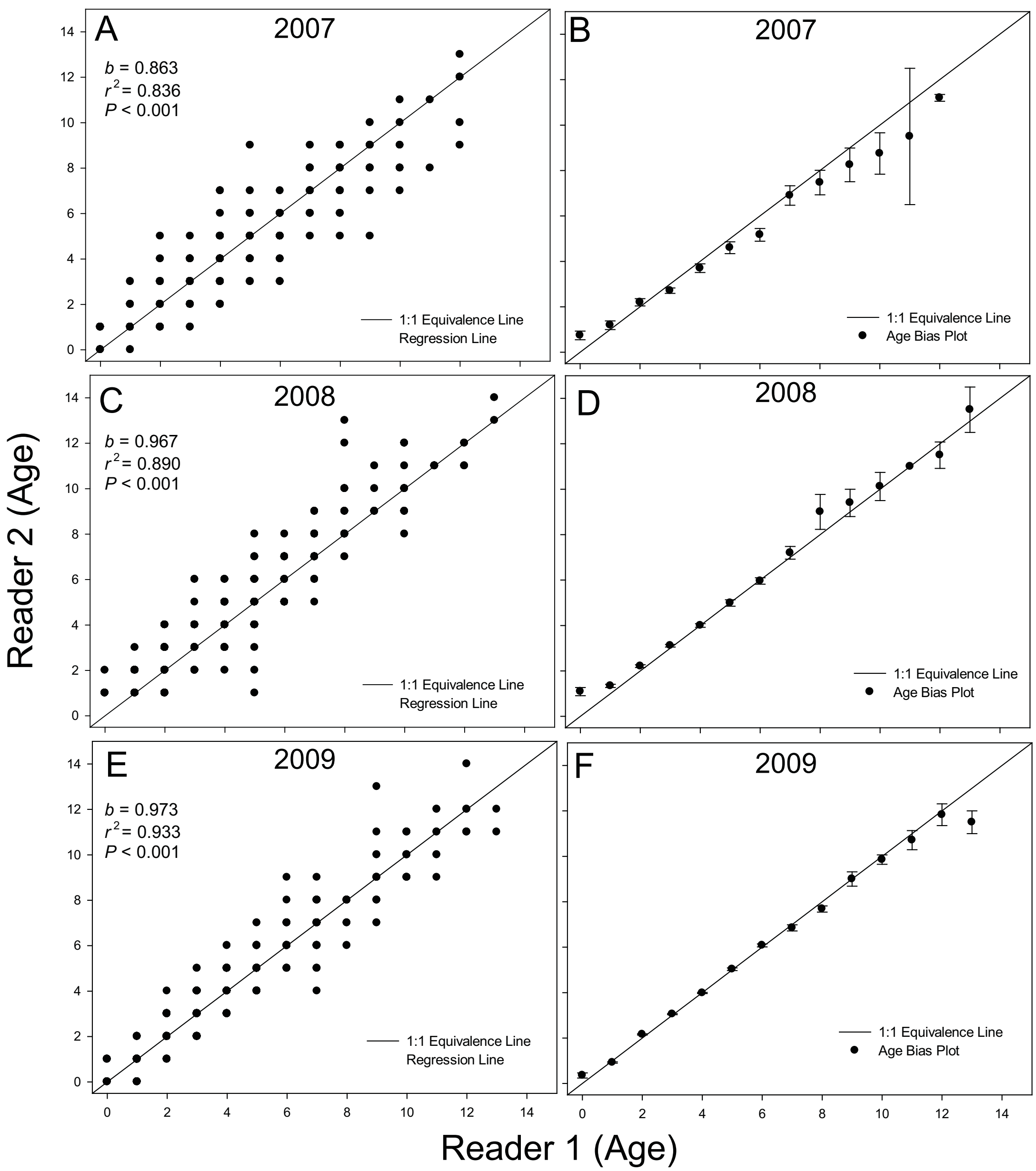

FIGURE 2. Scatter plot regressions and age-bias plots of channel catfish age estimates assigned by two readers for 2007 (A, B), 2008 (C, D), and 2009 (E, F) pectoral spines compared to a 1:1 equivalence line (b = regression slope).

estimations are observed for age-7 to age-10 channel catfish, with much greater bias for age-11 and older fish. Between-structure precision also appears to be low, with only 45% exact reader agreement (Table 1). However, precision dramatically increases for PA-1 and PA-2.

Removal, setting, image capturing, and reading times were significantly greater for otoliths compared to pectoral spines (Table 2). Though pectoral spine processing required additional steps (filling, popping, and cytosealing), overall processing time for pectoral spines (11.01 min $\pm$ 0.07) was signifi-

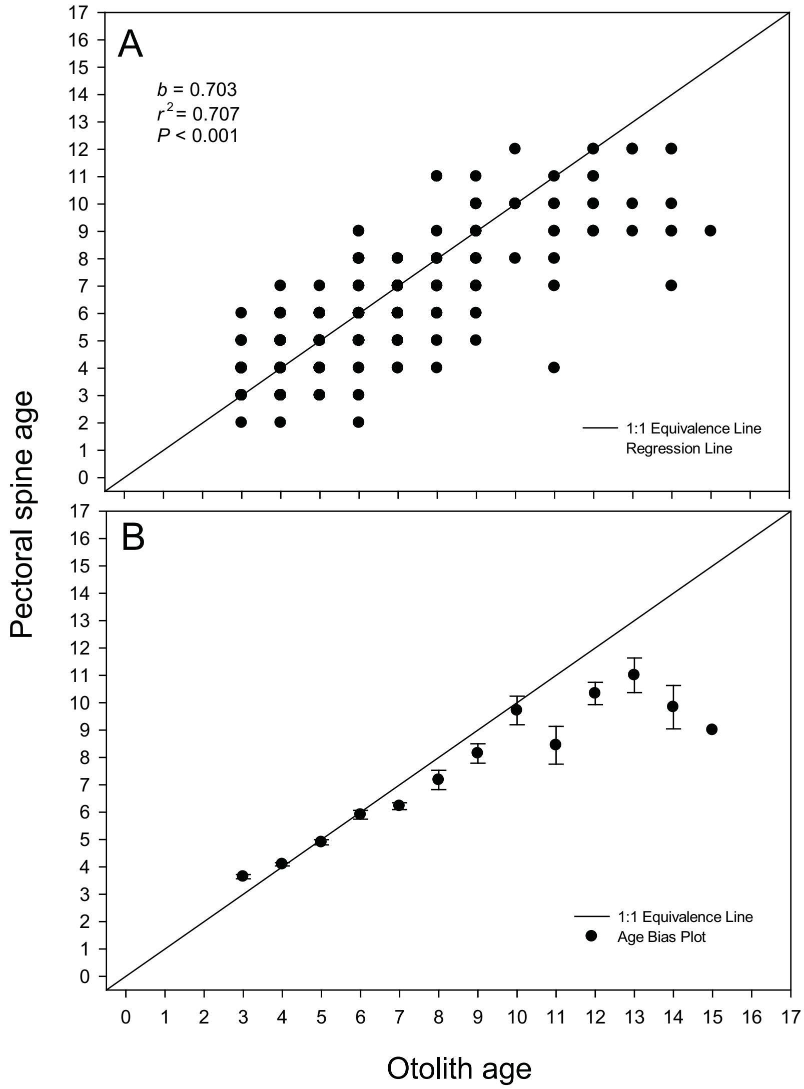

FIGURE 3. Scatter plot regression (A) and age-bias plots with 95% confidence intervals (B) of channel catfish age estimates assigned for otoliths and pectoral spines compared to a 1:1 equivalence line (b = regression slope).

cantly less than otolith processing time (12.26 min ± 1.09; $P = 0.04$; Table 2).

Discussion

Channel catfish otoliths generally provided greater precision and less reader bias compared to pectoral spines. However, processing time was less for pectoral spines compared to otoliths. Furthermore, precision and bias of age estimates from pectoral spines are positively influenced by increased reader experience. Age-specific bias was also observed for pectoral spines in relation to otoliths, especially for age-11 and older channel catfish.

Our findings of little bias and high precision for otolith age estimates support results from Buckmeier et al. (2002). However, high precision should not be mistaken for high accuracy. In fact, inaccurate age readings can be highly reproducible (Campana 2001). Nonetheless, high precision of age estimates obtained from valid structures provides some confidence in accuracy.

Experienced readers provided more precise and less biased pectoral spine age estimates for channel

TABLE 2. Mean and standard error of individual procedure times and total processing times (minutes) for pectoral spines and otoliths used for age estimation of channel catfish. An asterisk indicates mean processing time for pectoral spines is significantly different than otoliths for the specific procedure ($P < 0.05$).

	Pectoral spine		Otolith	
Procedure	Mean	SE	Mean	SE
Removal*	0.36	0.01	1.43	0.04
Setting*	1.43	0.13	2.77	0.23
Filling	0.33	0.03	–	–
Popping	1.03	0.15	–	–
Cutting/sanding*	5.56	0.18	2.71	0.49
Cytosealing	1.29	0.10	–	–
Image capturing*	0.77	0.10	4.82	0.31
Reading*	0.25	0.01	0.52	0.02
Total processing time*	11.01	0.07	12.26	1.09

catfish. Similar reader experience trends have been observed with otolith and scale age determinations for black crappie *Pomoxis nigromaculatus* and white crappie *P. annularis* (Ross et al. 2005). Therefore, we recommend that experienced readers be used for age estimation whenever possible. Quality control should also be conducted periodically during age and growth investigations (Campana 2001).

Pectoral spines overestimate young (age 3) channel catfish age in relation to otolith age estimates. Over-aging of younger channel catfish was also observed by Buckmeier et al. (2002), who suggested that annular marks composed of multiple rings led to overestimation. Holland and Peters (1992) also observed “false annuli” on pectoral spine cross sections of channel catfish collected from the Platte River, Nebraska. Therefore, overestimation of younger fish was likely due to interpreting false marks as true annuli.

We found that pectoral spines underestimated age of older channel catfish compared to otoliths. Nash and Irwin (1999) documented that pectoral spines underestimated adult flathead catfish *Pylodictis olivaris* age compared to otoliths. Underestimation of age using pectoral spines likely occurs due to expansion of the central lumen (Muncy 1959; Nash and Irwin 1999) and/or merging of outer annuli (Devries and Frie 1996), especially with older, slow-growing individuals. Pectoral spine age estimations for age-7 to age-10 channel catfish only underestimated fish age slightly (~1 year) compared to otoliths. This may suggest that the central lumen has expanded just past the first annulus in age-7 to age-10 channel catfish. However, higher variability in age estimates of age-11 and older fish suggests that the lumen has expanded to encompass two or more annuli and/or that annuli are merging near the margin. Bias and precision of pectoral spine age estimates can be problematic, especially with older fish. Conversely, otoliths have been validated as an accurate structure to age channel catfish (Buckmeier et al. 2002) in the southeastern United States. Our findings indicate that otolith age estimates are nonbiased and highly precise up to age-14 channel catfish, suggesting that otoliths are the aging structure of choice when feasible.

Most channel catfish populations are dominated by younger fish, with few individuals exceeding age 8 (Hubert 1999). Therefore, it may be possible to obtain accurate age estimates using pectoral spines for such populations. In some cases, sacrificing a limited number of fish from a population for otolith age reference could also improve pectoral spine age estimates (Campana 2001; Buckmeier et al. 2002; Michaletz et al. 2009).

All preparation times, besides cutting/sanding, were greater for otoliths compared to pectoral spines. Otolith preparation time has also been documented to be greater compared to other aging structure preparation times for striped bass *Morone saxatilis* (Welch et al. 1993), walleye *Sander vitreus* (Isermann et al. 2003), and yellow perch *Perca flavescens* (Vandergoot et al. 2008). However, these studies also indicated that reading time was less for otoliths compared to other structures, unlike our findings. Longer preparation and reading times for otoliths resulted in overall otolith processing time to be greater compared to pectoral spine processing time in our study. A significant difference in overall processing time should be noteworthy to resource managers seeking the most time and cost-efficient methods for aging channel catfish.

A difference of only a minute per structure could be substantial if age and growth sample sizes are in the hundreds or thousands.

Aging structures were collected from channel catfish for this study during spring, summer, and fall. Time of year samples were taken can influence results and lead to greater variability in age estimates. Therefore, our results are probably a conservative estimate of how close the hard parts and readers are in terms of agreement. Marginal increment analysis of channel catfish aging structures would be beneficial to steer timing of collections. Additionally, research is needed to validate age structures for older (>age 4) channel catfish. Specifically, investigations conducted in northern latitudes using fish from both lotic and lentic habitats would be beneficial in understanding bias, precision, and accuracy associated with age structures throughout the geographic range of channel catfish.

Our findings coupled with previous research on channel catfish aging illustrate that neither spines nor otoliths alone are ideal aging structures for all channel catfish investigations and emphasize the need for age validation. However, information herein can be used to make educated decisions. We recommend that managers making decisions about what aging structure to use in a particular situation (1) use previous data and/or anecdotal evidence to approximate age distribution of the population of interest and to determine whether sacrificing channel catfish is viable to sustain desired population characteristics, (2) determine experience level of readers for specific aging structures, (3) determine level of bias and precision needed for statistical analyses, and (4) determine amount of time and money allotted to complete specific aging duties.

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