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ARTICLE

Precision of Calcified Structures Used for Estimating Age of Chain Pickerel

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Abstract
With the exception of Northern Pike *Esox lucius*, there is a paucity of information on the precision of age estimates based on calcified structures for esocid species, especially Chain Pickerel *E. niger*. We estimated ages for 42 Chain Pickerel from Stevens Creek Reservoir on the Savannah River, South Carolina–Georgia. Ages were estimated using sectioned pelvic, pectoral, dorsal, and anal fin rays, scales, cleithra (sectioned and whole), and otoliths to assess precision between repeated readings (four readers). Precision was evaluated by calculating agreement among readers, CV, and mean absolute difference (MAD) in estimates between reader pairs. We tested for differences in precision of estimates among structures using linear mixed models with CV or MAD as the response. Finally, we used age bias plots to evaluate whether there were structure or reader-related systematic biases in estimates. Coefficient of variation and MAD were significantly related to structure type (CV: $P = 0.006$, MAD: $P = 0.001$) and mean estimated age (CV: $P < 0.001$, MAD: $P = 0.025$). Precision was poor for all structures and poorest for age-0 fish; however, all analyses indicated that otoliths provided the most precise estimates. We observed agreement in estimates among all readers for 40% of the otoliths examined and consensus agreement for 74% of the estimates. Coefficient of variation for otoliths declined from 67% for fish with a mean estimated age of 0 years to 5% for age-4 fish. Conversely, MAD for otoliths increased from 0.34 years for fish with a mean estimated age of 0 to 0.46 years for age-4 fish. Estimates from all structures, except pectoral rays, were negatively biased compared with estimates from otoliths. We recommend the use of otoliths for age estimation of Chain Pickerel, but managers and researchers should be cognizant of and evaluate the potential effects of variability in age estimates among readers on their analyses.

Age estimation of fish is necessary for the assessment of fish population dynamics (e.g., recruitment, growth, and mortality) and stock structure (Maceina et al. 2007). Age data are incorporated into age-structured population models (Maceina et al. 2007) and used by fisheries managers to guide decisions about harvest strategies and conservation programs (Spurgeon et al. 2015). The most commonly used method for age estimation is interpretation of calcified structures (Faust et al. 2013). Using calcified structures can result in errors due to discrepancies in estimated age between readers and discrepancies between true and estimated ages (Dortel et al. 2013). Errors in processing and interpretation of structures can lead to biases (Dortel et al. 2013), and inaccurate estimation can result in invalid population assessment or poor management decisions (Beamish and McFarlane 1983; Maceina et al. 2007; Hamel et al. 2016; Tyszko and Pritt 2017). To resolve this issue, precision and accuracy of calcified

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structures should be estimated (Spurgeon et al. 2015), and fisheries models should account for uncertainty and errors in age estimation (Dortel et al. 2013).

Structures used for age estimation should be accurate and precise (Casselman 1979; Campana et al. 1995; Blackwell et al. 2016; Phelps et al. 2017). In a review of 1,351 articles about validation of age estimates from calcified structures of various fishes, Spurgeon et al. (2015) found that the Northern Pike *Esox lucius* was the only esocid that validation studies have been conducted for and those studies focused on periodicity of annuli formation. Historically, viewing whole cleithra has been believed to provide the most reliable age estimates for esocids (Casselman 1979); however, age estimates from otoliths have similar or better precision than estimates from whole cleithra for Northern Pike (Faust et al. 2013; Oele et al. 2015; Blackwell et al. 2016). Although several studies (see Phelps et al. 2017) have focused on age estimation of Northern Pike, fewer studies have investigated the reliability of calcified structures for age estimation of other esocids, especially Chain Pickerel *E. niger*. Saila (1956) used scales to estimate ages for Chain Pickerel, and he observed that uncertainty in age estimates increased with age. To our knowledge, there have been no attempts to validate or estimate the precision of age estimates based on calcified structures from Chain Pickerel.

To address the lack of information about precision of age estimates for Chain Pickerel, we estimated ages using sagittal otoliths, whole cleithra, sectioned cleithra, scales, and sectioned anal, pectoral, pelvic, and dorsal fin rays for fish collected from Stevens Creek Reservoir on the Savannah River, South Carolina–Georgia. Ages were estimated by four readers to identify which structure was most precise. Identifying the most precise structure, assuming accuracy, for estimating the age of Chain Pickerel will improve our understanding of Chain Pickerel biology and provide guidance for managers interested in Chain Pickerel populations.

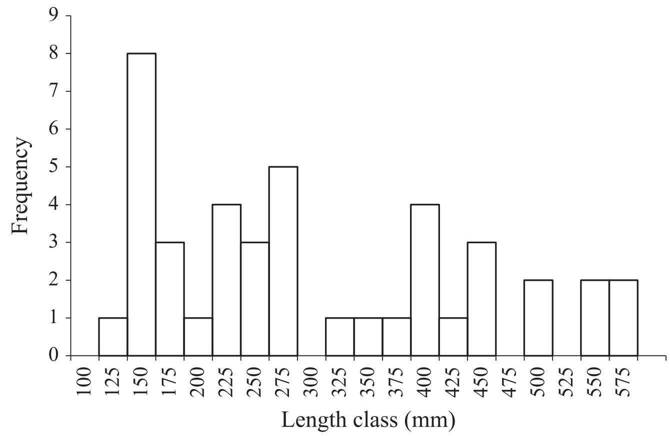

FIGURE 1. Length frequency distribution for 42 Chain Pickerel collected from Stevens Creek Reservoir on the Savannah River, South Carolina–Georgia.

METHODS

Sample collection.— Chain Pickerel were collected in September and November 2016 by means of boat electrofishing. Length and weight data were recorded for each fish, and fish were frozen for later processing. Total length of fish ranged from 117–571 mm, and the mean length was 291 mm (Figure 1). After thawing, we removed sagittal otoliths, scales, cleithra, and anal, dorsal, pelvic, and pectoral fins from each fish and allowed them to dry in paper coin envelopes. Scales were collected from an area dorsal to the lateral line and directly anterior to the dorsal fin (Laine et al. 1991; Blackwell et al. 2016). Cleithra were removed following the methods of Casselman (1979). Anal, dorsal, pelvic, and pectoral fins were clipped as close to the body as possible and at a right angle to the fin rays using diagonal cutting pliers (Fischer and Koch 2017). We used diagonal cutting pliers to prevent fin rays from splitting, because this may occur when cutting blades that cross each other (e.g., scissors) are used.

Processing.— We mounted whole otoliths measuring <4 mm long on glass slides using Crystalbond 509 (Ted Pella, Redding, California) because they were too small to be sectioned. Mounted otoliths were then polished using 600-grit and 1,000-grit sandpaper. After initial polishing, we viewed the otoliths with oblique transmitted light under an Olympus SZX-10 Zoom Stereomicroscope (Olympus Corporation, Tokyo) equipped with an Olympus SZX2-ILLT illumination stand and Olympus DP-27 camera. Otoliths were either viewed directly through the microscope or on a computer monitor with Olympus cellSens Standard software to see whether more polishing was required. We then flipped the samples and repeated the polishing procedure on the reverse side to ensure even polishing. Otoliths > 4 mm long were set in epoxy (EpoKwick, Buehler, Lake Bluff, Illinois) using low-density polyethylene (LDPE) wells. Next, we cut a 0.6-mm transverse section through the core of each otolith (Faust et al. 2013; Blackwell et al. 2016) with an IsoMet 1000 precision low-speed saw (Buehler, Lake Bluff, Illinois) equipped with two blades spaced 0.6 mm apart with spacers made from acetate overhead projector film. Finally, we mounted sectioned otoliths on slides using Crystalbond 509.

Cleithra were placed in near-boiling water for about 20 s to loosen remaining flesh, and loosened flesh was removed with tweezers and by lightly scraping the bone with a scalpel or wire brush (Casselman 1979). We allowed cleaned cleithra to air dry and then returned them to their respective labeled coin envelopes for storage. Once age estimation was complete for all whole cleithra, we removed the heel section of one cleithrum from each fish using a circular Dremel 4000 variable-speed rotary tool (Dremel, Racine, Wisconsin) (Blackwell et al. 2016) and mounted them in epoxy using the same protocol used for larger otoliths. We cut 0.6-mm sections through the origin with an IsoMet 1000 precision low-speed saw using the same double-blade technique that was used for larger otoliths.

Scales were placed in water and allowed to soak before excess skin was removed with a toothbrush and tweezers. Then, we dried the scales on paper towels before placing them back in their respective envelopes. We removed the fourth ray from anal fins and the second ray from the pelvic fins, because preliminary analyses indicated that they provided the most accurate estimates of age from their respective fins in the closely related Muskellunge *E. masquinongy* (D. P. Crane, unpublished data). Because the dorsal fin is similar in structure to the anal fin, we also removed the fourth ray from the dorsal fin. We removed the second ray from the pectoral fin because of the pectoral fin's structural similarity to the pelvic fin. Water was used to loosen excess skin from each fin ray, and excess tissue was removed using tweezers and a scalpel. Afterwards, the distal ends of individual fin rays were inserted into nondrying modeling clay. We then encased rays with a ~3-cm-long section of a plastic drinking straw (~1 cm diameter), sealing the base of the straw with modeling clay. Once positioned, the straws were labeled with the fish's identification (ID) and structure and filled with epoxy, and the epoxy was allowed to cure for 24 h (modified from Koch and Quist 2007). Fin rays that were too thin to remain upright in modeling clay were set using the same procedure described for setting otoliths > 4 mm. Next, we used an IsoMet 1000 precision low-speed saw to cut 1.2-mm sections from the proximal end of each sample. The proximal end of the ray was sectioned because sections close to the base of fin rays provide more reliable age estimates than sections from the distal portion of the ray (Koch et al. 2008; Watkins et al. 2015).

Age estimation.— Four readers independently estimated the ages of Chain Pickerel using otoliths, whole and sectioned cleithra, scales, and sectioned anal, dorsal, pelvic, and pectoral fin rays. Reader experience with age estimation ranged from limited to 1.5 years experience, and all readers were trained to estimate the ages from each structure. Readers practiced viewing structures with a zoom stereo microscope and associated software before beginning data collection. Ages were estimated by counting annuli in each structure (Figures 2, 3). To avoid bias, fish ID was not provided until an estimated age was recorded

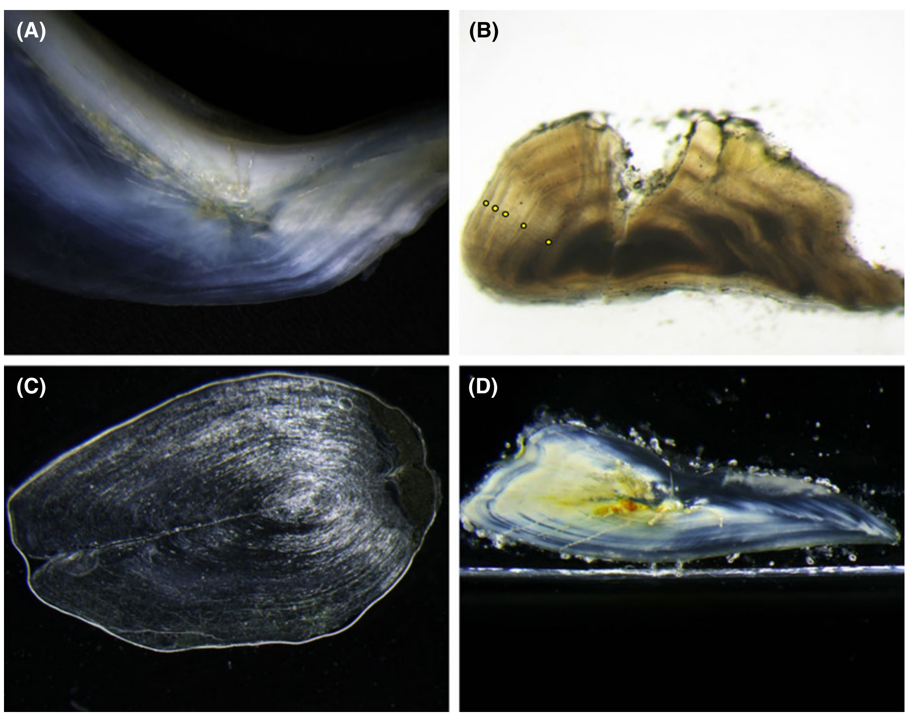

FIGURE 2. Photographs of calcified structures used for age estimation of Chain Pickerel. All structures were from the same female (TL = 565 mm, weight = 1,198 g). This fish was estimated to be age 5 based on consensus age estimated from its sectioned otolith. **(A)** Heel of cleithrum; annuli were not easily discernible on the blade of the cleithrum, so we focused on the heel, **(B)** sagittal otolith, **(C)** scale, and **(D)** sectioned cleithrum. Yellow points indicate counted annuli on the sectioned otolith.

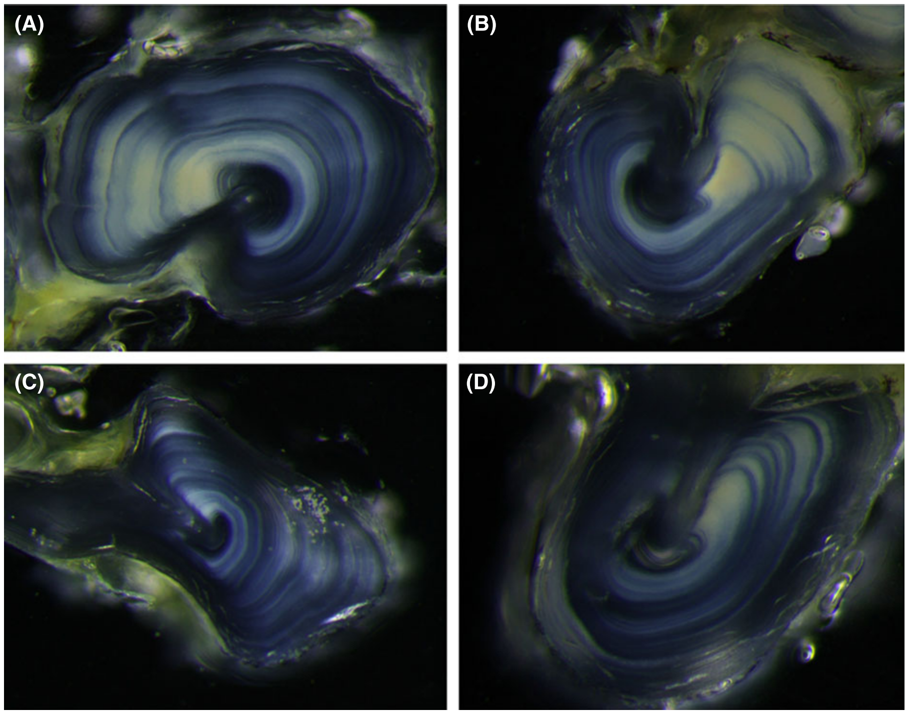

FIGURE 3. Photographs of calcified structures used for age estimation of Chain Pickerel. All structures were from the same female (TL = 565 mm, weight = 1,198 g). This fish was assumed to be age 5 based on consensus age estimated from its sectioned otolith. **(A)** Anal fin ray, **(B)** dorsal fin ray, **(C)** pectoral fin ray, and **(D)** pelvic fin ray.

for a particular sample. The orders that structures and samples from a given structure were examined were haphazardly selected (i.e., the order of fish that ages were estimated for varied among structures). Before examination, otoliths and sectioned fin rays were wiped with immersion oil (type A) to remove any residue from the polishing and sectioning processes. Samples were then placed on a slide, covered with a drop of immersion oil, and viewed using a zoom stereo microscope (transmitted light). Readers were able to either view samples on a computer monitor using the previously mentioned camera and software or directly through the eyepieces, because the readers had their own visual preference that they believed would provide the most reliable interpretations. Otoliths were viewed using the oblique setting on the microscope base, whereas fin rays were viewed with darkfield, oblique, or a combination of settings to count annuli based on the illumination technique that provided the best contrast between translucent and opaque zones in the structure. Whole cleithra were placed in a black matte petri dish filled with water and viewed under an overhead light to count annuli. Readers typically focused on the heel of cleithra because the blade frequently had large translucent zones that made annuli difficult to discern. Sectioned cleithra were viewed in the same manner as that described for sectioned fin rays. Scales and a drop of water were pressed between two glass slides and were viewed with a combination of oblique and darkfield illumination. Annuli were counted where "cutting over" was observed within circuli (Laine et al. 1991).

Analysis.— We analyzed age estimates for 42 Chain Pickerel using R statistical software within RStudio (2016, version 1.0.153; R Core Team 2016, version 3.3.1) and Microsoft Excel (Microsoft, Redmond, Washington). First, we used the FSA package in R (Ogle 2017; Function: agePrecision, version 0.8.13) and Excel to calculate a series of descriptive statistics for each structure including: (1) percent of samples with 100% agreement among all readers, (2) percent of samples with consensus agreement (agreement among at least three of four readers), (3) percent of reader pairs that agreed on estimated age, and (4) percent of reader pairs with estimates within 1 and 2 years. Next, we calculated fish- and structure-specific CV (100·SD/mean estimated age) and mean absolute difference (MAD) in estimated age between reader pairs. The CV provided a measure of precision that was scaled to the mean estimated age, and MAD provided an estimate of average absolute disagreement between readers in the original unit of years. Then, we used the lme4 package (Bates et al. 2015; Function:lmer) to fit linear mixed models to determine the effects of calcified structure type on CV and MAD. Coefficient of variation or MAD was the

response variable; calcified structure type, mean estimated age, and an interaction between structure type and mean estimated age were the fixed effects; and fish ID was incorporated as a random intercept within each model. We included mean estimated age in the models to test the assumptions that CV and MAD did not vary with age and to determine whether mean values for CV and MAD (averaged across ages) could be reported or whether age-dependent values for CV and MAD were necessary. We tested the effects of structure type, mean estimated age, and the interaction between structure type and mean estimated age on CV and MAD using the Anova() function (type III Wald
F-test with Kenward–Rogers estimated df) in the car package (Fox and Weisberg 2011). Statistical significance was determined based on an alpha level of 0.05.

To evaluate whether there were structure- or reader-related systematic biases in age estimates, we created age-bias plots (Campana et al. 1995) for each reader pair and between structures using the FSA package (Ogle 2017; Function: ageBias, version 0.8.13). Between-structure bias plots were created by comparing mean estimated ages from each structure to the consensus age (agreement for three of four reader estimates) for the structure that provided the most precise age estimates. To create between-reader age-bias plots, estimates from the first reader (x-axis) were plotted against mean estimated ages and 95% CIs for the corresponding samples from the second reader (y-axis) with a 1:1 agreement line.

RESULTS

One-hundred percent agreement among all readers was highest for otoliths (40%), followed by whole cleithra (24%), then sectioned cleithra (21%), and was lowest for anal and pelvic fin rays (9.52%; Table 1). Consensus agreement was greatest for pectoral rays (76%), otoliths (74%), and scales (71%), and lowest for dorsal rays (40%). One-hundred percent agreement between pairs was highest for otoliths (64%), followed by pectoral fin rays (52%), then sectioned cleithra (50%), and was lowest for pelvic fin rays (38%; Table 1). The average percent agreement within 1 year was highest for otoliths (97%), followed by sectioned cleithra (88%), then scales (84%), and was lowest for pelvic fin rays (75%; Table 1). The average percent agreement within 2 years was highest for otoliths and sectioned cleithra (99%), followed by dorsal fin rays (96%), then pelvic fin rays (92%), and was lowest for whole cleithra (86%; Table 1).

Coefficient of variation for estimated ages was significantly related to structure type ($F = 2.8806$, df = 7, df residuals = 285, $P = 0.006$) and mean estimated age ($F = 18.0687$, df = 1, df residuals = 319, $P < 0.001$), but there was not a significant interaction between structure type and mean estimated age ($F = 1.7674$, df = 7, df residuals = 287, $P = 0.094$). Coefficient of variation decreased with mean estimated age for all structures (Figure 4). Because CV was dependent on structure type and age, we used structure-specific models to estimate CVs for ages 0–4 (Figure 4). Coefficient of variation could not be reliably estimated for age-5 and older fish because mean estimated age was ≥5 for few samples. Estimates from dorsal fin rays had the lowest mean CV for fish with a mean estimated age of 0–1 (age 0 = 53%, age 1 = 48%), but the CV for otoliths was comparable among fish estimated to be age 1 (51%), and estimates from otoliths resulted in the lowest CVs for fish with estimated ages of 2–4 (age 2 = 36%, age 3 = 20%, age 4 = 5%). With the exceptions of estimates from dorsal rays for fish estimated

TABLE 1. Summary statistics for eight calcified structures used to estimate the ages of Chain Pickerel collected from the Stevens Creek Reservoir on the Savannah River, South Carolina–Georgia: 100% agreement among all readers, consensus agreement (agreement among at least three of four readers), percent of agreement between reader pairs, and percent of reader pairs with estimates within 1 and 2 years.

Structure	n	100% agreement among all readers	Consensus agreement	100% agreement between pairs	±1 year	±2 years
Otoliths	42	40.48%	73.81%	63.89%	97.22%	98.81%
Sectioned cleithra	42	21.43%	61.90%	50.00%	88.10%	98.81%
Cleithra	42	23.81%	64.29%	41.27%	78.57%	86.11%
Dorsal fin (fourth ray)	42	19.05%	40.48%	44.44%	82.14%	96.43%
Pectoral fin (second ray)	42	19.05%	76.19%	51.59%	76.98%	92.46%
Scales	42	11.90%	71.43%	45.63%	83.73%	90.48%
Anal fin (fourth ray)	42	9.52%	59.52%	39.68%	76.98%	91.67%
Pelvic fin (second ray)	42	9.52%	45.24%	37.70%	74.60%	92.86%

to be age 0–2 and scales for fish estimated to be age 4, estimates from lethal structures had lower CVs than did estimates from nonlethal structures.

Mean absolute difference between reader pairs had a positive relationship with mean estimated age (F = 10.9897, df = 1, df residuals = 320, P = 0.001), and differences in MAD between structure types were dependent on mean estimated age (F = 2.3388, df = 7, df residuals = 288, P = 0.025). Mean absolute difference increased with mean estimated age for all structures (Figure 4). As was done for CV, we used structure-specific models to estimate MADs for ages 0–4 (Figure 4). Estimates from pectoral fin rays had the lowest MAD for fish with a mean estimated age of 0 (0.29), but MAD was similar for estimates from dorsal rays (0.30), otoliths (0.34), and cleithra (0.34). Estimates from otoliths had the lowest MADs for fish with estimated ages of 1–4 (age 1 = 0.37, age 2 = 0.40, age 3 = 0.43, age 4 = 0.46).

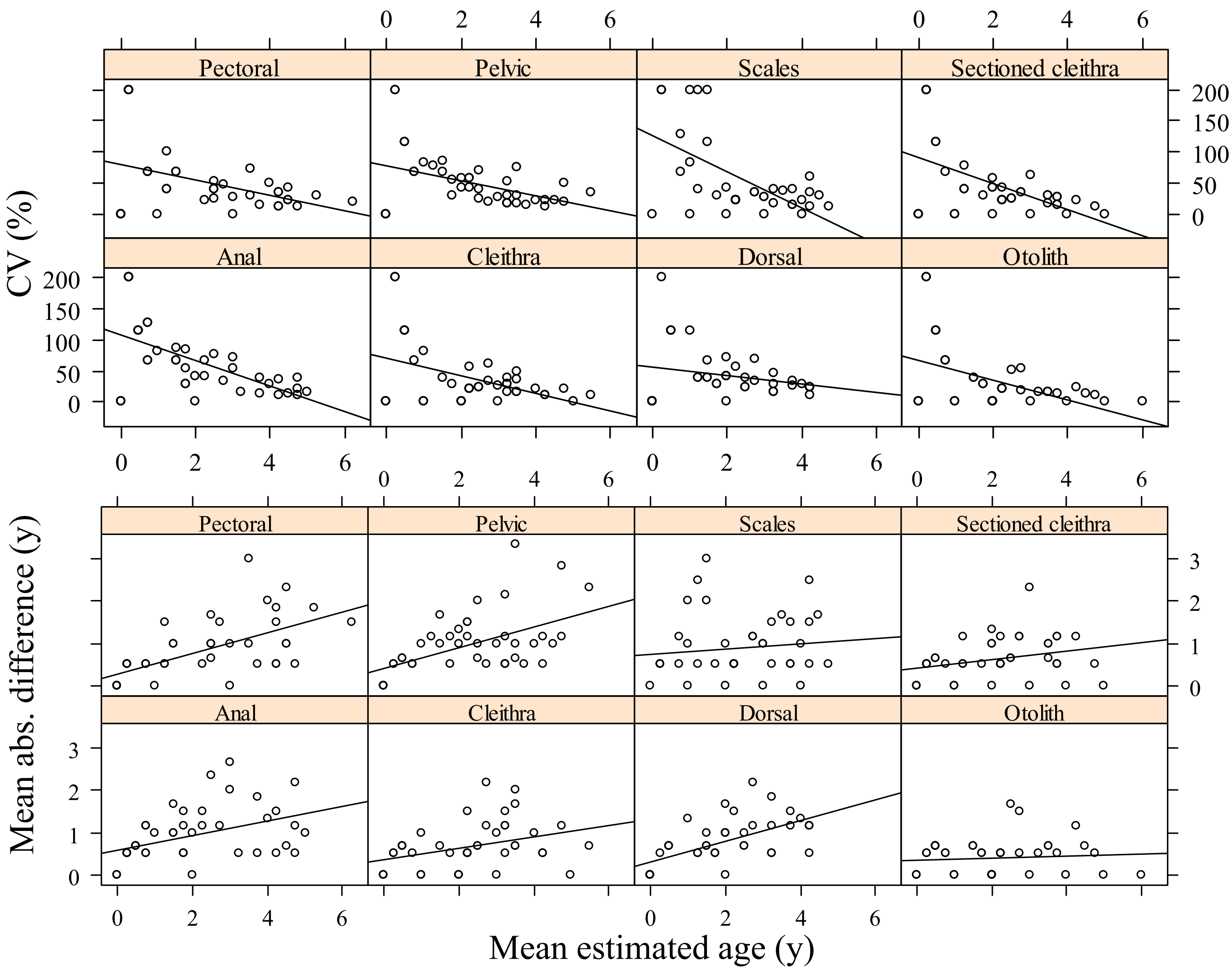

FIGURE 4. Scatterplots and fitted lines indicating the relationships between mean estimated age (MAE; years) and CV (%) and MAE and mean absolute difference (MAD; years) in estimates between reader pairs for Chain Pickerel from Stevens Creek Reservoir on the Savannah River, South Carolina–Georgia. Trend lines are based on linear mixed-effects models for each structure. Trend lines for CV are described by the equations: CV pectoral fin rays = 77.7203 − 11.6927MEA; CV pelvic fin rays = 74.8472 − 11.3031MEA; CV scales = 123.2556 − 28.4379MEA; CV sectioned cleithra = 88.8411 − 20.2977MEA; CV anal fin rays = 106.7908 − 19.9863MEA; CV cleithra = 69.4072 − 13.6533MEA; CV dorsal fin rays = 53.3794 − 5.8533MEA; CV otoliths = 66.8172 − 15.5307MEA. Trend lines for MAD are described by the equations: MAD pectoral fin rays = 0.2872 + 0.2429MEA; MAD pelvic fin rays = 0.4192 + 0.2466MEA; MAD scales = 0.7424 + 0.0664MEA; MAD sectioned cleithra = 0.4184 + 0.1016MEA; MAD anal fin rays = 0.5850 + 0.1790MEA; MAD cleithra = 0.3392 + 0.14105MEA; MAD dorsal fin rays = 0.29562 + 0.2516MEA; MAD otoliths = 0.3366 + 0.0304MEA.

Mean absolute difference was generally comparable among estimates from nonlethal structures, and estimates from nonlethal structures resulted in greater MADs than estimates from lethal structures. For nonlethal structures, estimates from pectoral and dorsal rays tended to have the lowest MADs for fish with mean estimated ages of 0–2, but scales had the lowest MAD for fish that were estimated to be age 3–4.

Age-bias plots revealed trends in bias between structures and readers (Figure 5; also see figures in the Supplement available in the online version of this article). Because estimates from otoliths were the most precise, between-structure age-bias plots were created using consensus ages from otoliths as the reference values. Consensus age was observed for 31 of 42 estimates (74%) from otoliths and ranged from ages 0 to 6. With the exception of estimates from pectoral fin rays, estimates from all other structures had a negative bias for fish with otolith-based consensus estimates of age 5 or age 6 (Figure 5). Large 95% CIs for estimated ages based on fin rays

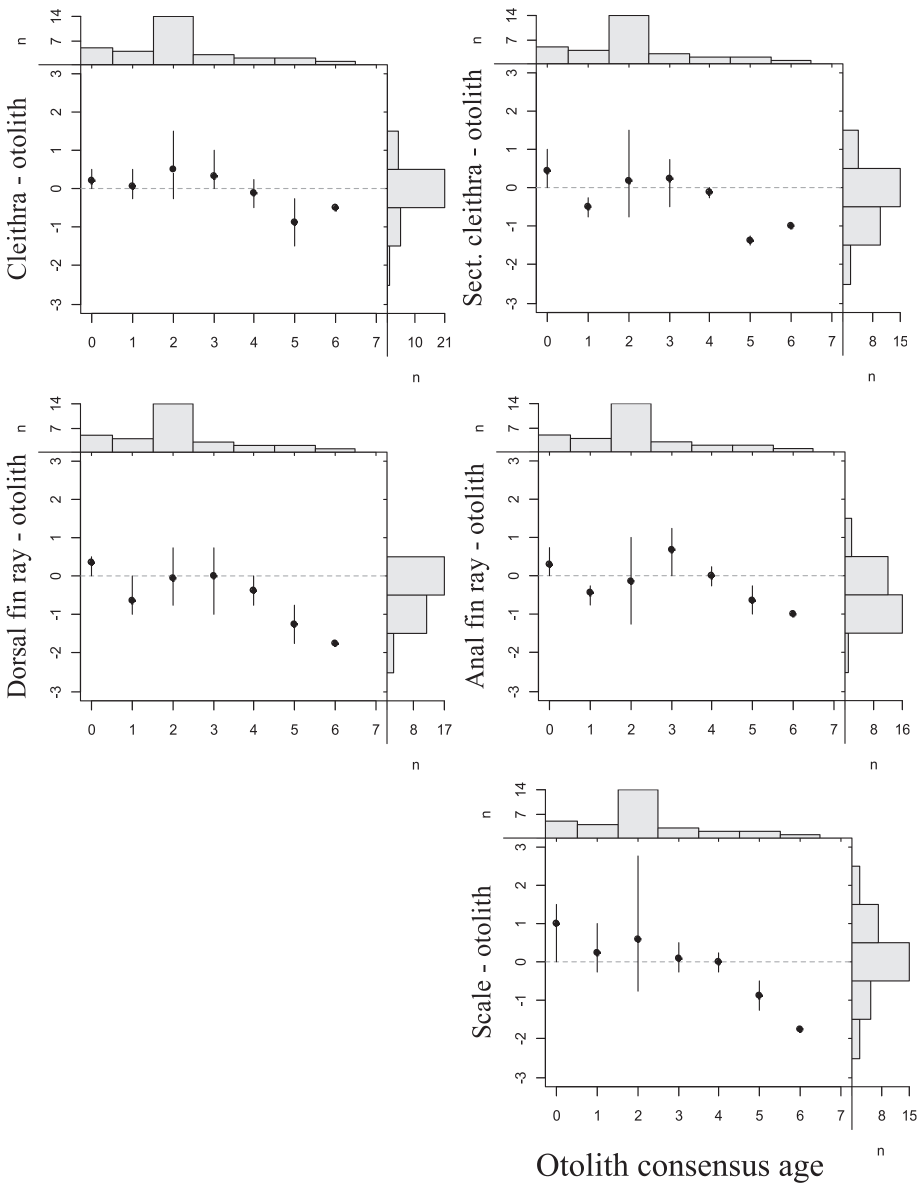

FIGURE 5. Age-bias plots between structures used to estimate age of Chain Pickerel from Stevens Creek Reservoir on the Savannah River, South Carolina–Georgia. Age-bias plots were created based on the consensus age (agreement among three of four readers) for otoliths (x-axis) and the difference between estimates from the structure of interest and otoliths (y-axis). Otoliths were used as the reference values because estimates from otoliths were the most precise of the structures examined. Error bars represent 95% CIs, and histograms represent the distribution of samples for each axis.

indicated substantial variability in age estimates between readers compared with ages estimated from otoliths, cleithra, and sectioned cleithra (see the Supplement). No bias was observed between readers when using otoliths to estimate age (see the Supplement). However, disagreement between readers increased with increased fish age for most structures, especially scales.

DISCUSSION

Whole cleithra have served as the most recognized calcified structure used for estimating the age of esocids (Casselman 1979; Faust et al. 2013); however, our cumulative results indicated that otoliths provide the most precise estimates of age for Chain Pickerel. Similarly, two recent studies found that precision of age estimates from otoliths were similar to or more precise than estimates of age for Northern Pike based on cleithra (Oele et al. 2015; Blackwell et al. 2016). Although we determined that otoliths provided the most precise estimates, CVs for samples with mean estimated age < 4 years were substantially greater than the suggested reference CV of 5% (Campana 2001). Similarly, MADs between reader pairs were small for otoliths (0.33–0.46 years for ages 0–4), but small absolute differences in age estimates have the potential to substantially affect growth estimates for young or relatively short-lived fishes such as Chain Pickerel. Chain Pickerel have a maximum recorded age of about 9 years (Rohde et al. 2009), and the maximum estimated age that was agreed upon by all readers in our study was 6 years. Due to sample size constraints, we were unable to assess the effects of uncertainty in our age estimates on population characteristics such as growth, mortality, and recruitment, but future work should focus on quantifying the consequences of uncertainty and error of age estimates.

Most of the readers in this study had limited age-estimation experience, which may have contributed to relatively poor precision of estimates across structures. However, it is difficult to directly compare estimated CVs and MADs from this study with other studies because most studies report average CV (averaged across all samples) or MAD, whereas, we had to estimate CV and MAD at specific ages because they were significantly related to mean estimated age. We are unaware of how common it is for CV or MAD to be significantly related to mean estimated age, but we suggest that researchers test the assumption that they do not vary with age before reporting mean values in order to avoid making erroneous conclusions or invalid comparisons with other study results. For example, if CVs or MADs are related to age, estimates of these metrics may be affected by the age distribution of a sample. Therefore, to make valid comparisons among studies, CVs and MADs would need to be compared in the context of age distributions.

Whole cleithra were relatively easy to read, but similar to otoliths, age estimates were imprecise. Problems with estimating age by using whole cleithra arose when it took several minutes to view the structure because samples would absorb water and become translucent. This was easily corrected by removing the sample from the water and allowing it to dry before viewing again, but it was time consuming. We believe that sectioning cleithra made samples easier to read; however, sectioning cleithra did not improve precision compared with viewing the whole cleithra, which is contrary to the findings of Blackwell et al. (2016) for Northern Pike. When viewing sectioned cleithra, a yellow residue was observed in every sample (Figure 2D), which partially obscured early growth bands. Polishing samples with 1000-grit sandpaper rarely helped remove the residue.

Overall, age estimates from nonlethal structures were less precise than estimates from lethal structures. Distinguishing annuli within scales was difficult compared with the other structures examined, and similar to findings from other studies investigating the use of calcified structures for estimating age of fishes (Fitzgerald et al. 1997; Zymonas and McMahon 2009; Acre et al. 2017), we observed poor precision of ages estimated from scales. Additionally, scales resulted in underestimation of age and a younger maximum age compared with otoliths, which was likely due to erosion or reabsorption on outside edges of scales. Age estimates from fin rays were less precise than those from otoliths or cleithra, but were comparable with those from scales. Commonly, there was poor visibility along the edges of rays, which made it difficult to reliably estimate ages for samples that were presumed to be from older fish (Figure 3). Also, the degree of contrast between opaque and translucent zones in fin rays was variable among samples. Although readers agreed that the shape of pectoral fin rays made it difficult to locate and distinguish between annuli along the outer edges of sections, pectoral fin rays had CVs and MADs that were comparable with estimates from other nonlethal structures, had the highest 100% agreement among readers for nonlethal structures (tied with dorsal rays), had the highest consensus agreement among all structures, and was the only structure that did not result in estimates that were negatively biased compared with estimates from otoliths. Therefore, if nonlethal sampling is desirable, we recommend the use of the second pectoral ray for age estimation.

When lethal sampling is acceptable, we recommend the use of otoliths for age estimation of Chain Pickerel. Otoliths provided the most precise estimates of the structures examined, and readers agreed that both sectioned (i.e., larger) and polished (i.e., smaller) otoliths were the easiest structures to read. A common problem with otoliths was that the central portion surrounding the nucleus frequently had a large opaque area that potentially obscured the first annulus. It was fairly easy to over-polish smaller otoliths when trying polish this area to increase transparency and reveal any annuli in this region. We recommend collecting extra otoliths to be used for practicing polishing methods before processing structures to be used in a study.

Not knowing the true age of the fish collected in this study prevented us from properly validating the use of otoliths for age estimation of Chain Pickerel; therefore, future research assessing age estimation of Chain Pickerel should consider using fish of known age to estimate accuracy of age estimates from otoliths. Studies should also account for the time taken to age each sample, duration of processing, and how confident readers are in their estimates (Fitzgerald et al. 1997). Finally, fish used in this study were collected from the southern portion of the Chain Pickerel's geographic distribution. Chain Pickerel from more northern latitudes may experience different patterns of opaque and translucent zone formation due to differences in the duration of the growing season across the distribution of the species. By further assessing the precision and validity of age-estimation techniques, across a broader geographic range, fisheries managers can make more informed management decisions for Chain Pickerel fisheries.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.