

SPECIAL SECTION: ANGLING FOR DINOSAURS

Relative Bias and Precision of Age Estimates among Calcified Structures of Spotted Gar, Shortnose Gar, and Longnose Gar

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Abstract

Recreational angling for gars (family Lepisosteidae) has become more popular in recent years; however, the fundamental understanding of their population dynamics needed for effective management and conservation is lacking. Age data are essential for describing population dynamic rate functions, but few studies have addressed the selection of ideal calcified structures for estimating age in gars. We collected Spotted Gars *Lepisosteus oculatus*, Shortnose Gars *L. platostomus*, and Longnose Gars *L. osseus* from 12 Illinois water bodies to assess the relative bias and precision of age estimates derived from branchiostegal rays, pectoral fin rays, cleithra, and sagittal otoliths. Age assignments differed among these calcified structures for all three species. Branchiostegal rays underestimated the age of young fish and overestimated the age of old fish relative to all other structures. Pectoral fin rays consistently underestimated age relative to other structures and produced the lowest mean and maximum age estimates. Although there was low relative bias between readers for all structures, age assignments showed greater variability between readers for old age-classes when age estimates were derived from cleithra and otoliths. Between-reader precision was highest using pectoral fin rays, whereas cleithra and otoliths generated lower coefficients of variation and percent agreement values. These findings reveal a need to improve or modify structure processing methods to increase readability when cleithra and otoliths are used for age estimation. Given these results, future validation studies should target branchiostegal rays and pectoral fin rays to determine the most accurate calcified structure for aging gars.

Historically, the four species of gars native to Illinois (Longnose Gar *Lepisosteus osseus*, Shortnose Gar *L. platostomus*, Spotted Gar *L. oculatus*, and Alligator Gar *Atractosteus spatula*; Page and Burr 2011) were considered a nuisance by anglers and fisheries managers due to their reputation for competing with and consuming more desirable sport fishes (Caldwell 1913; Gowanloch 1939; Bonham 1941; Holloway 1954; Suttkus 1963; Scarnecchia 1992). Alligator Gars have been extirpated from Illinois since the 1960s (Burr et al. 1996; Poly 2001), presumably

due to habitat loss and overharvest. Spotted, Shortnose, and Longnose gars are still found in slow-moving waters of Illinois' large rivers, backwaters, and tributaries (Page and Burr 2011). In recent years, gars have been recognized for their ecological role in aquatic ecosystems in maintaining diversity and creating balance within the aquatic food web (Scarnecchia 1992). In addition, gars are now more frequently targeted and captured by recreational anglers and bowfishers in many parts of the United States (Ferrara 2001; Bennett et al. 2015; Binion et al. 2015). Due to

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the emerging interest in gar fishing, sustainable management strategies must be initiated to avoid overharvest. However, little is known about the population dynamics of gars in Illinois, in large part due to the paucity of age data needed to assess growth, mortality, and recruitment as well as to predict the influence of harvest.

Fisheries managers commonly use age estimates obtained from calcified structures to evaluate dynamic rate functions to aid in the development of sustainable management practices (Quist et al. 2012). Collecting accurate age data is a high priority because these data are crucial to understanding the ecology and management of fish species (Ricker 1975). Numerous calcified structures have been evaluated, and the most accurate structure has been found to vary among species (Maceina et al. 2007). For example, sagittal otoliths provide the most accurate age estimates of Channel Catfish *Ictalurus punctatus* (Buckmeier et al. 2002), pectoral fin rays are generally preferred for Shovelnose Sturgeon *Scaphirhynchus platyrhynchus* (Jackson et al. 2007), and cleithra are used to age Northern Pike *Esox masquinongy* and Muskellunge *E. lucius* (Faust et al. 2013). Branchiostegal rays are historically the calcified structure most widely used to age gars (Netsch and Witt 1962; Redmond 1964; Klaassen and Morgan 1974; Johnson and Noltie 1997; Love 2004; Murie et al. 2009; Sutton et al. 2009; Kelley 2012). Previous research assumed that branchiostegal rays provide accurate age estimates based on the high precision between readers (Netsch and Witt 1962; Murie et al. 2009); however, this structure has not been successfully validated for any species of gar. In their effort to validate Alligator Gar ages using branchiostegal rays, Buckmeier et al. (2012) found that accuracy could not be directly assessed because oxytetracycline (OTC) was not visible in the structure. However, ages derived from branchiostegal rays produced underestimates of age relative to validated ages derived from sagittal otoliths (Buckmeier et al. 2012). Sagittal otoliths have been used to age Longnose Gars, Alligator Gars, and Spotted gars (Ferrara 2001; DiBenedetto 2009; Glass et al. 2011; Buckmeier et al. 2012; Smylie et al. 2016), with mixed results. Whole sagittal otoliths of Alligator and Longnose gars were unreadable (Ferrara 2001), whereas sagittal otoliths ground along the transverse plane were validated for aging Alligator Gars in Texas (Buckmeier et al. 2012). Similarly, sagittal otoliths were embedded in epoxy resin and transverse sections were used to age Longnose Gars in estuaries in South Carolina (Smylie et al. 2016). Age estimates obtained from sectioned pectoral fin rays of Alligator Gars could not be evaluated for accuracy due to the lack of visible OTC marks, but ages were comparable to those from validated sagittal otoliths in fish up to 1,200 mm TL (\leq age 6) (Buckmeier et al. 2012). Glass et al. (2011) compared age estimates from ground otoliths, branchiostegal rays, and sectioned

pectoral fin rays of Spotted Gars in Rondeau Bay (Lake Erie). Although the accuracy of age assignments was not evaluated, pectoral fin rays were found to be more precise than branchiostegal rays, and the authors concluded that pectoral fin rays are a useful nonlethal method for aging the species (Glass et al. 2011). Although several calcified structures have been used to determine the age of gars, few studies have directly compared calcified structures in the same individuals to evaluate relative bias (Ferrara 2001; Glass et al. 2011; Buckmeier et al. 2012). In the absence of validation, the relative bias and precision among calcified structures can provide the initial evaluation needed to develop accurate age-related metrics for management (Casselman 1983).

Fisheries managers often rely on dynamic rate functions (such as those for growth and mortality) developed from age data to establish appropriate regulations for sustainable fisheries (Quist et al. 2012). Inaccurate age data can produce variable growth and mortality curves, which will inevitably alter management practices. As interest in gars grows among recreational anglers, identifying appropriate calcified structures for accurate and precise age determination is a fundamental first step to effective management of this sport fishery. The objective of this study was to document the procedures used to collect and prepare branchiostegal rays, pectoral fin rays, cleithra, and sagittal otoliths from Spotted, Shortnose, and Longnose gars from Illinois and to determine the relative bias and precision of age estimates among structures and readers.

METHODS

Fish collection.—Gars were collected from twelve Illinois water bodies from April 13, 2015, to June 27, 2016, using gill nets, fyke nets, and boat electrofishing (alternating and direct current). Fish were also collected from state bowfishing tournaments (Illinois Bowfishing Association, Bowfishing Association of America, and Tri-State Bowfishers) and from individual anglers who captured them via bowfishing and rod and reel. Whole specimens were immediately placed on ice and transported to the laboratory for processing.

Calcified structure processing.—Each fish was identified to species, weighed to the nearest gram, and measured to the nearest millimeter total length. Branchiostegal rays, pectoral fin rays, sagittal otoliths, and cleithra were extracted from each specimen to estimate age. The largest left and right branchiostegal rays were removed, placed in boiling water in 1-min increments to loosen excess tissue, and scrubbed clean. This process was repeated several times until all tissue was removed. After 15 samples, we increased the boiling time in 1-min increments and found that branchiostegal rays could be sufficiently cleaned after they were boiled for 3 min. Branchiostegal rays were dried

in labeled coin envelopes for at least 24 h before being viewed under a microscope. The left, anterior-most pectoral fin ray was separated from the rest of the fin and removed from the body at the most proximal end, similar to the procedure described by Koch et al. (2008) and Quist et al. (2012). It is crucial that fin rays be cut at the base because the first annulus may only be present in the most proximal section (Koch et al. 2008; Quist et al. 2012). Fin rays were dried in labeled coin envelopes for at least 24 h before being set in epoxy resin (Epofix cold-setting embedding resin #1232; Electron Microscopy Sciences, Hatfield, Pennsylvania) using 1.5-mL centrifuge tubes as molds, similar to the procedure outlined by Koch and Quist (2007). The hardened epoxy containing the fin ray was removed from the centrifuge tube mold, and at least three transverse sections (0.75 ± 0.10 mm) were cut from the most proximal end (Koch et al. 2008; Quist et al. 2012) with a Buhler Isomet low-speed saw (Model 11-1280-160; Buehler, Lake Bluff, Illinois). The left cleithrum was removed using tin snips, placed in boiling water in 1-min increments to loosen excess tissue, and then scrubbed clean. This process had to be repeated several times to sufficiently clean the cleithra, so after 15 samples we increased the boiling time in 1-min increments and determined that 5 min of boiling time was adequate to clean the structure. Cleithra were dried in labeled coin envelopes for at least 24 h before being read. The sagittal otoliths were removed, wiped clean, and suspended in epoxy resin using PELCO 111 LDPE Flat Embedding Molds (Ted Pella). Transverse sections (0.75 ± 0.05 mm) through the primordia (or nucleus) were cut using a Buehler Isomet low-speed saw similar to the procedure used by Smylie et al. (2016); the cuts were made so that the surface of each completed section was perpendicular to the anterior-posterior axis.

Pectoral fin ray sections, otolith sections, whole branchiostegal rays, and whole cleithra were placed in a dark dish, immersed in mineral oil, and viewed using a Fein Optic FZ6-ILST Stereo Zoom Dissection Microscope at $6.7\text{--}67.5\times$ magnification (small otoliths and pectoral fin rays were viewed at up to $135\times$) with reflected light. Two readers aged each structure independently without knowledge of the fish's length. Disagreements in age estimates between readers were reconciled; if agreement could not be reached, the fish was excluded from the analysis.

Data analysis.—Age bias plots were used to evaluate the relative bias of the consensus age estimates among structures, whereas independent age assignments were used for comparisons between readers. For each pairwise comparison among structures, the average age estimated using one structure was plotted against the full range of corresponding consensus age estimates based on a second structure. To construct between-reader age bias plots, the average age assignments from reader 2 were plotted

against the assigned ages by reader 1. Plots with points clustering around a 1:1 line indicated that the age assignments were unbiased (Campana et al. 1995).

To evaluate the precision (i.e., repeatability) of age estimates among calcified structures and between readers, the average coefficient of variation (CV; $100 \times \text{SD}/\text{mean}$), percent agreement (i.e., exact agreement), and percent agreement within 1 year were derived (Campana et al. 1995). Coefficients of variation were calculated for each individual across structures and again between readers, and then a nonparametric Kruskal–Wallis test with a Tukey's honestly significant difference (HSD) post hoc test were used to determine whether CV values differed among structures (Jackson et al. 2007; Oele et al. 2015). Percent agreement and CV measurements were calculated in version 0.8.7 of the Fisheries Stock Analysis package (Ogle 2016).

Age distributions were constructed for each calcified structure to visualize the differences in age estimates among structures. Because fish were collected from multiple water bodies in Illinois, these data do not reflect the age structure of a specific population and therefore dynamic rate functions could not be determined for this study.

RESULTS

Relative Bias and Precision among Calcified Structures

Consensus age estimates were biased among calcified structures for all three gar species in that pectoral fin rays, otoliths, and cleithra overestimated the ages of young fish and underestimated the ages of old fish relative to branchiostegal rays (Figures 1–3). Pectoral fin rays consistently underestimated age relative to otoliths and cleithra for Spotted Gars (Figure 1), Shortnose Gars (Figure 2), and Longnose Gars (Figure 3). For Spotted and Shortnose gars, otoliths underestimated the ages of fish younger than ages 6 and 4, respectively, relative to cleithra and overestimated the ages of fish older than age 10 (Figures 1 and 2). For Longnose Gars, age estimates assigned using otoliths were unbiased relative to those from cleithra until age 12 (Figure 3).

Precision among structures was poor (CV > 14.5%; percent agreement < 39.2%) for all three species (Figures 1–3). The lowest CV was between age estimates derived from the branchiostegal rays and cleithra of Spotted Gars (CV = 14.5%; Figure 1), whereas the highest was between estimates from the pectoral fin rays and otoliths of Longnose Gars (CV = 30.6%; Figure 3). The highest percent agreement among structures was between the pectoral fin rays and cleithra of Longnose Gars (39.2%), which increased to 74.5% for ages within 1 year (Figure 3).

The widest age distributions for Spotted Gars (2–24 years; Figure 4) and Shortnose Gars (0–16 years; Figure 5) were estimated using branchiostegal rays, whereas

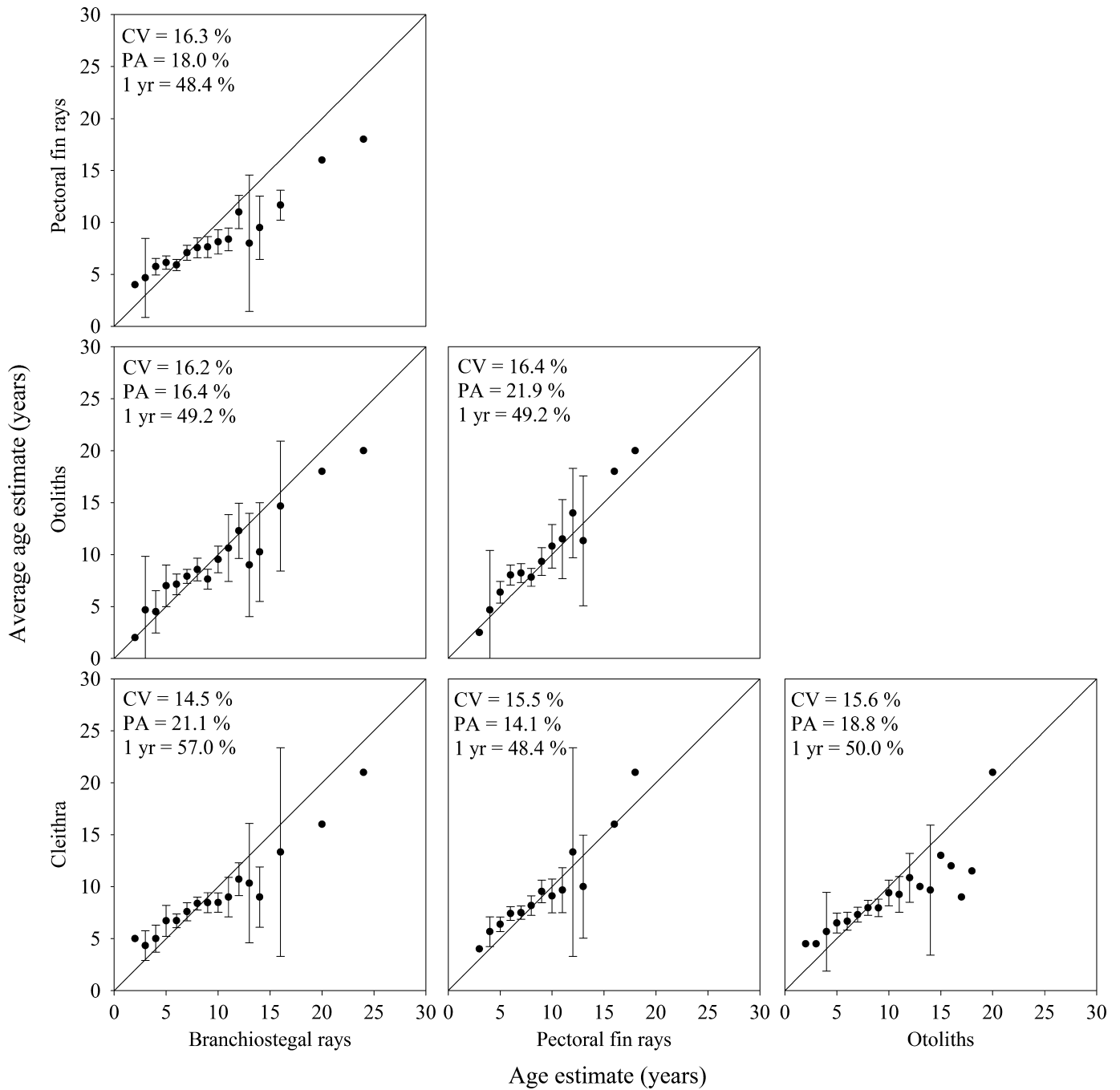


FIGURE 1. Relative age bias plots among structures using consensus age estimates from calcified structures of Spotted Gars ($n = 128$). Error bars represent 95% confidence intervals around the mean ages assigned by the structure on the y-axis for all fish assigned a given age by the structure on the x-axis. The solid lines indicate equivalence of the assigned ages. Numerical values for the precision of the structure comparisons are shown with the individual plots (CV = the coefficient of variation, PA = percent agreement, and 1 year = percent agreement within 1 year).

otoliths provided the widest age distribution for Longnose Gars (0–28 years; Figure 6). The estimates of mean age for each species varied among structures, with pectoral fin rays consistently providing the lowest mean and maximum ages (Figures 4–6).

Relative Bias and Precision between Readers

Overall, minimal relative bias was detected between readers for Spotted, Shortnose, and Longnose Gar structures. Age estimates assigned using branchiostegal rays and pectoral fin rays produced the least relative bias between

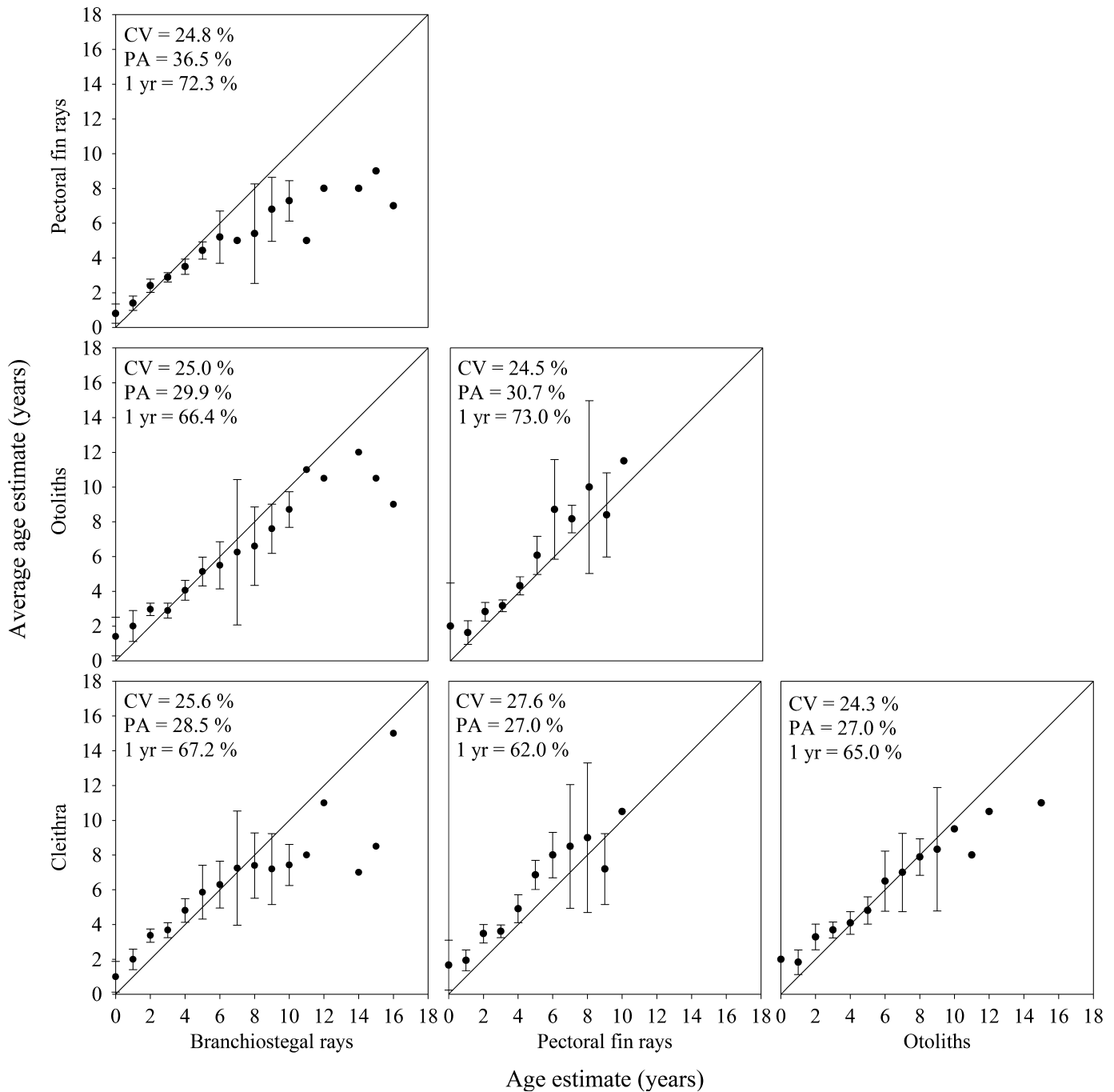


FIGURE 2. Relative age bias plots among structures using consensus age estimates from calcified structures of Shortnose Gars ($n = 137$). See Figure 1 for additional details.

readers, whereas ages were less comparable between readers using otoliths and cleithra (Figures 7–9). The greatest relative bias between readers occurred using the otoliths of Longnose Gars, in which reader 2 consistently underestimated the ages of fish relative to reader 1 (Figure 9).

For all three species, precision estimates between readers varied from average to poor depending on the

structure used. Age assignments had poor percent agreement between readers ($\leq 65.0\%$) for all gar structures (Figures 7–9). Agreement within 1 year was also relatively low between readers for cleithra and otoliths ($\leq 80.4\%$) for all three species; it was highest in Spotted Gars (87.5%; Figure 7) and Shortnose Gars (92.7%; Figure 8) using pectoral fin rays and highest in Longnose Gars using

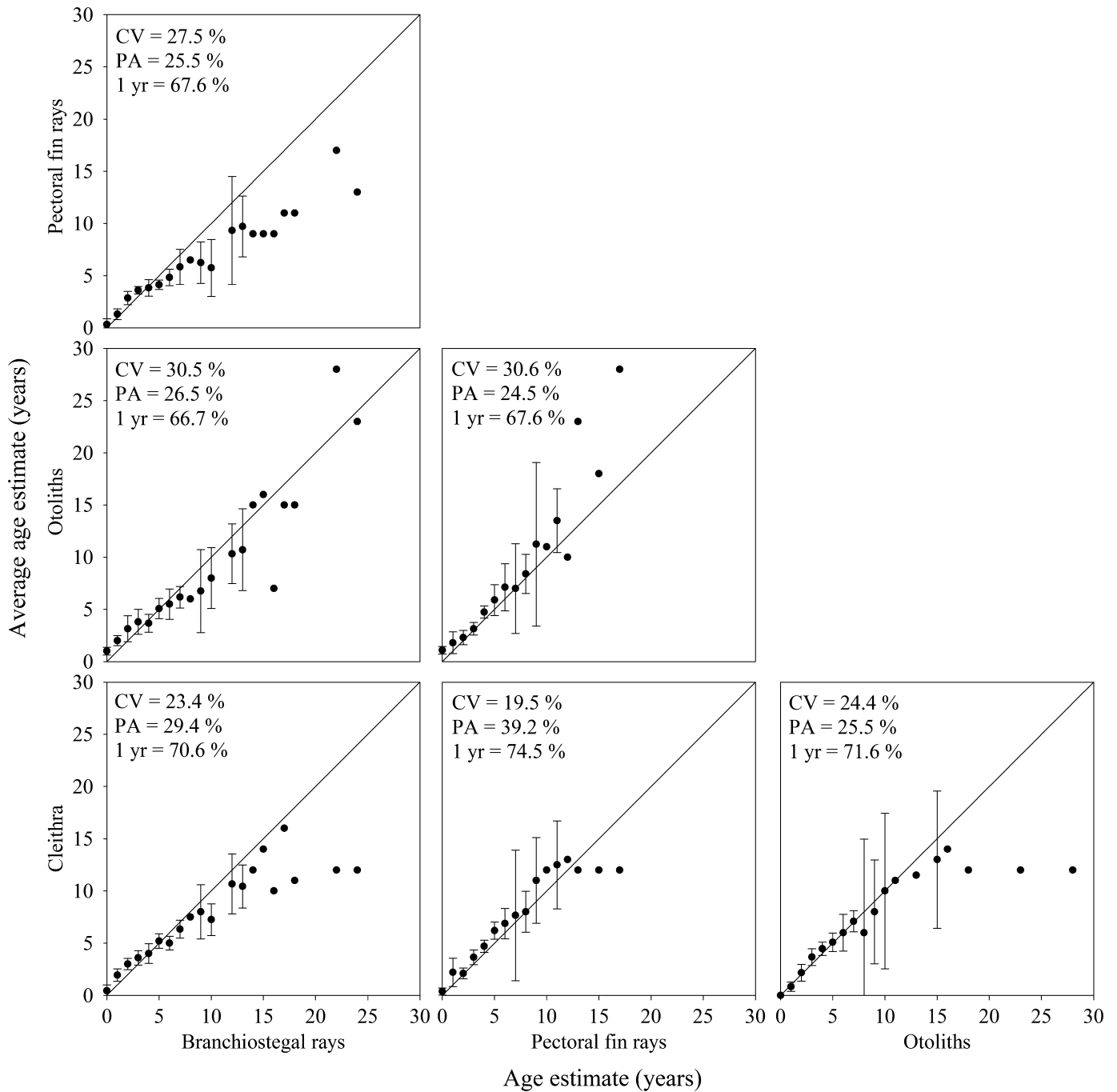


FIGURE 3. Relative age bias plots among structures using consensus age estimates from calcified structures of Longnose Gars ($n = 102$). See Figure 1 for additional details.

branchiostegal rays (90.2%; Figure 9). Coefficients of variation between readers were lowest in Spotted Gars (Figure 7) and generally higher for Shortnose Gars (Figure 8) and Longnose Gars (Figure 9). Pectoral fin rays produced the lowest CVs between readers for Spotted Gars (CV = 6.2%; Figure 7), Shortnose Gars (12.2%; Figure 8), and Longnose Gars (10.5%; Figure 9). The Kruskal–

Wallis test indicated that there were significant differences among structures in CVs between readers for all three species (Figure 10). The pectoral fin rays of Spotted Gars generated lower CVs between readers than did cleithra and otoliths (Tukey's HSD test; $P < 0.001$), whereas the pectoral fin rays of Shortnose Gars generated lower CVs between readers than cleithra, but not otoliths ($P < 0.05$;

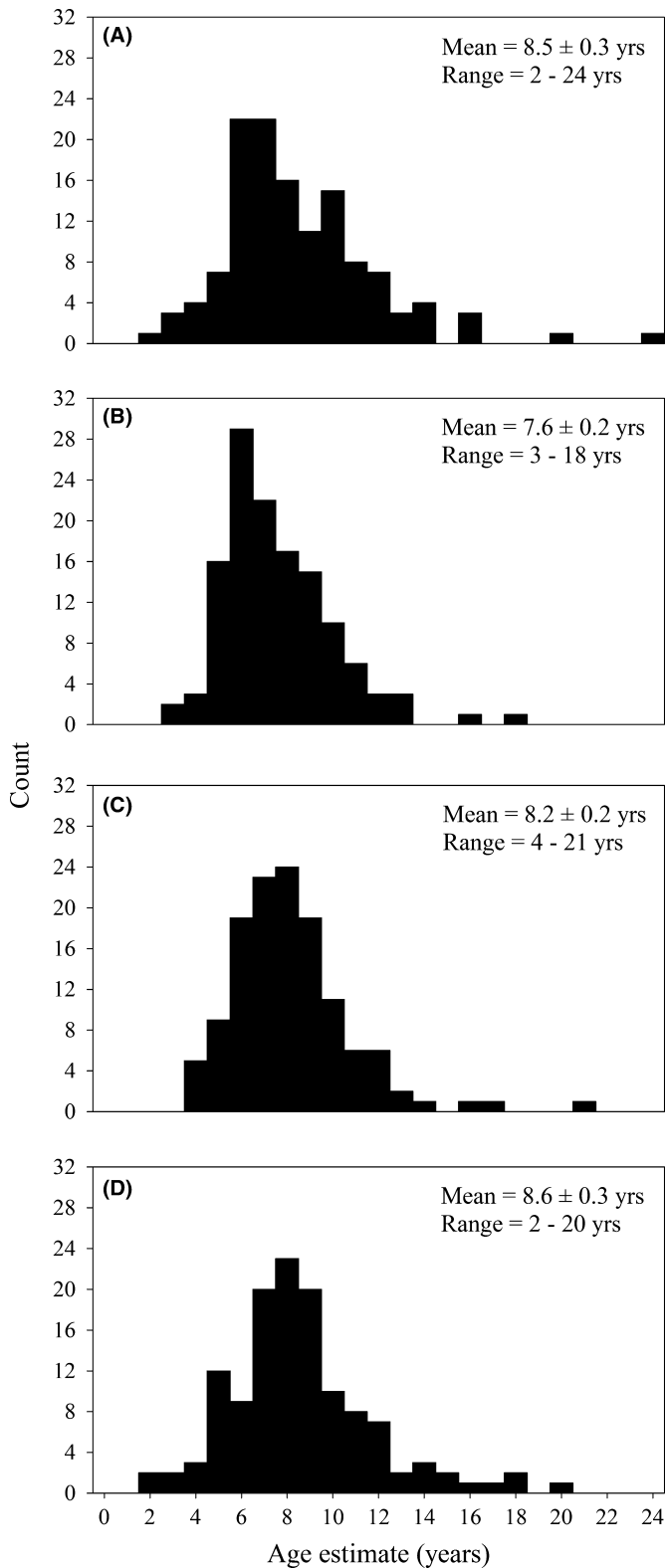


FIGURE 4. Age distribution plots using consensus age estimates from (A) branchiostegal rays, (B) pectoral fin rays, (C) cleithra, and (D) otoliths of Spotted Gars ($n = 128$). Means \pm SEs and ranges of age estimates are also shown.

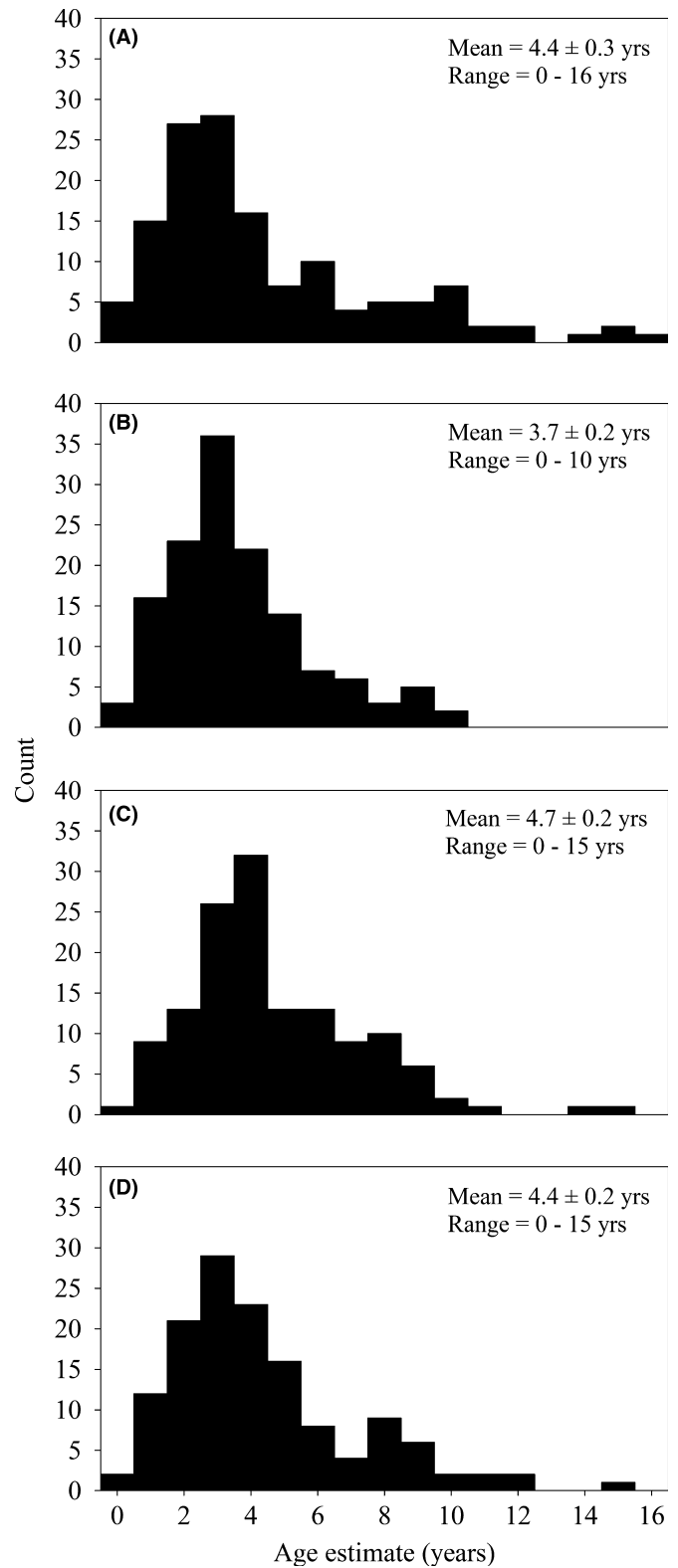


FIGURE 5. Age distribution plots using consensus age estimates from (A) branchiostegal rays, (B) pectoral fin rays, (C) cleithra, and (D) otoliths of Shortnose Gars ($n = 137$). Means \pm SEs and ranges of age estimates are also shown.

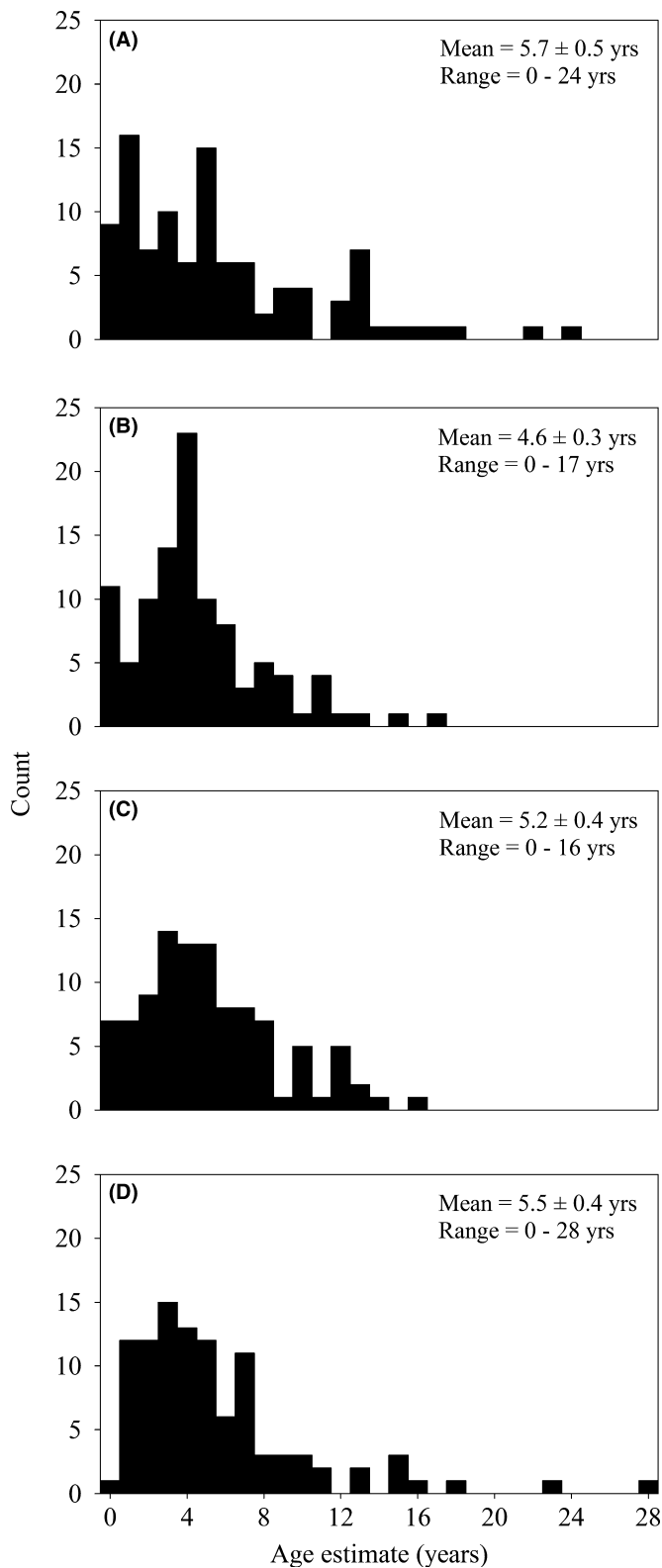


FIGURE 6. Age distribution plots using consensus age estimates from (A) branchiostegal rays, (B) pectoral fin rays, (C) cleithra, and (D) otoliths of Longnose Gars ($n = 102$). Means \pm SEs and ranges of age estimated are also shown.

Figure 10). The coefficients of variation between readers using the branchiostegal rays and pectoral fin rays of Longnose Gars were lower than those for otoliths ($P < 0.001$) but comparable to those for cleithra ($P > 0.05$). Branchiostegal rays and pectoral fin rays generated similar CVs between readers relative to each other for all three species ($P > 0.05$; Figure 10).

DISCUSSION

Relative bias was high and precision was low among the calcified structures of Spotted, Shortnose, and Longnose gars, indicating that the choice of calcified structure for aging gars is important for accurate age estimation. However, age estimates from branchiostegal rays, pectoral fin rays, sagittal otoliths, and cleithra have not been validated, so it is unclear which structure produces the most accurate age estimates. Discrepancies among structures are a concern because age-based dynamic rate functions for gar populations may differ depending on the structure used for age determination. Despite the common use of branchiostegal rays as an aging structure for gars (Netsch and Witt 1962; Klaassen and Morgan 1974; Johnson and Nolte 1997; Love 2004; Murie et al. 2009; Sutton et al. 2009; Kelley 2012), we found that this structure underestimates the ages of young fish and overestimates the ages of old fish relative to age assignments from the pectoral fin rays, sagittal otoliths, and cleithra of gars collected in Illinois. Branchiostegal rays from older specimens became opaque making it difficult to view annuli, which likely contributed to more relative bias between readers of older age classes in our study. Problems estimating the age of older fish using branchiostegal rays have been reported for large Alligator Gars and Longnose Gars (Ferrara 2001; Brinkman 2008; Buckmeier et al. 2012). Pectoral fin rays were deemed useful to age Spotted Gars in Rondeau Bay based on the high precision of the age estimates derived from them relative to those from branchiostegal rays (Glass et al. 2011); however, in our study pectoral fin rays consistently underestimated age relative to other structures and produced the lowest mean and maximum ages for all three species. Underestimation of age using pectoral fin rays has also been shown for Alligator Gars older than age 6 (Buckmeier et al. 2012), White Suckers *Catostomus commersonii* older than age 6 (Sylvester and Berry 2006), and Lake Sturgeon *Acipenser fulvescens* (Rossiter et al. 1995). The use of inaccurate age estimates is problematic and can influence population models and lead to management strategies that are detrimental to fish populations (Beamish and McFarlane 1995). Identifying the relative biases in age estimates among calcified structures can inform the use of population models until validation studies can determine the accuracy of each structure and identify the most appropriate structure or structures for aging gars.

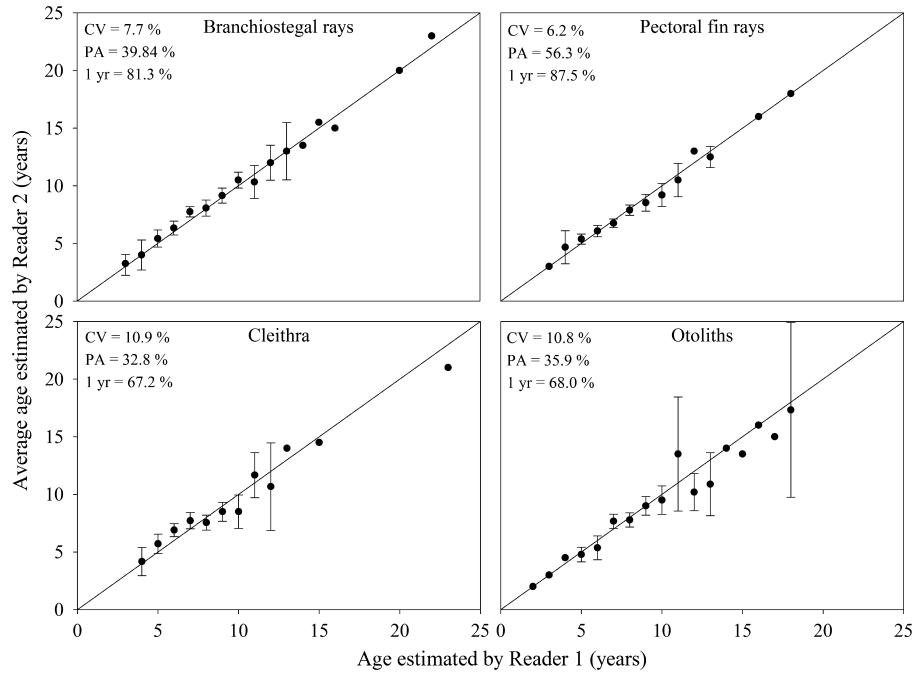


FIGURE 7. Relative age bias plots between readers for age estimates from calcified structures of Spotted Gars ($n = 128$). The error bars represent 95% confidence intervals around the average age assigned by reader 2 for fish assigned a given age by reader 1. The solid lines indicate complete equivalence in age estimates between readers. Precision values (%) for between-reader comparisons are shown with the individual plots.

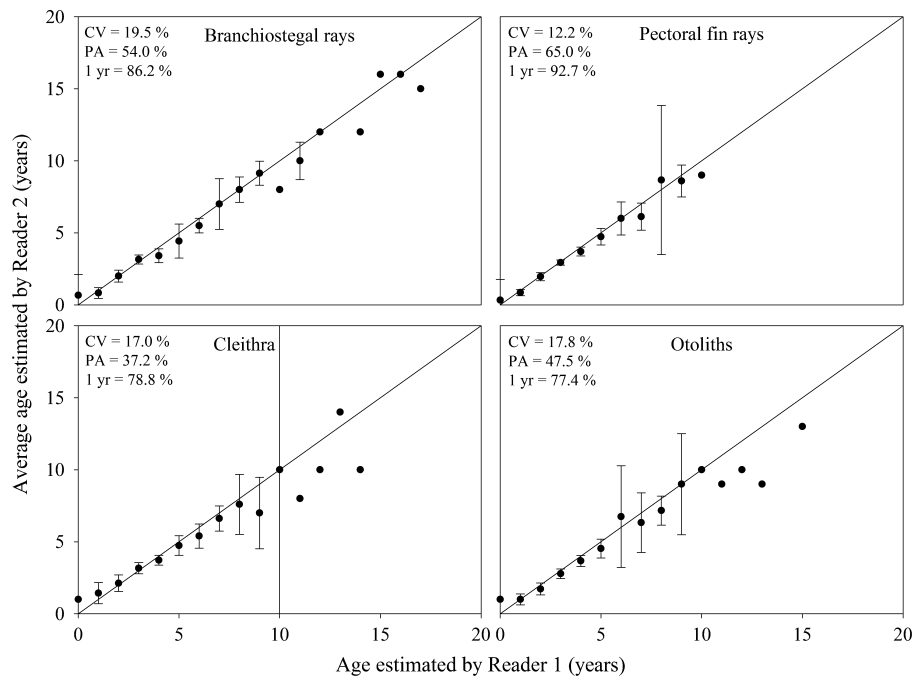


FIGURE 8. Relative age bias plots between readers for age estimates from calcified structures of Shortnose Gars ($n = 137$). See Figure 7 for additional details.

Branchiostegal rays and pectoral fin rays produced more precise age estimates between readers than cleithra and otoliths, with pectoral fin rays generating the lowest

CVs among calcified structures for all three gar species. However, based on Campana (2001), who suggests a target value of $\leq 5\%$, the CV values in our study are

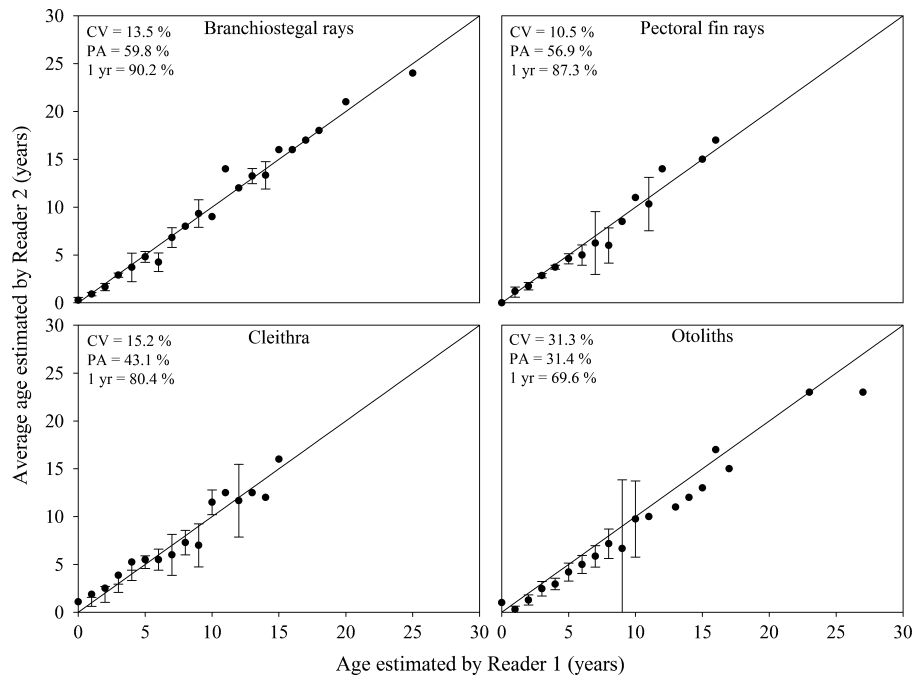


FIGURE 9. Relative age bias plots between readers for age estimates from calcified structures of Longnose Gars ($n = 102$). See Figure 7 for additional details.

considered average for Spotted Gars and poor for Shortnose and Longnose gars. The precision between readers basing age estimates on branchiostegal rays and pectoral fin rays was similar to those reported for Spotted Gars in Lake Erie (Glass et al. 2011). Agreement in age estimates between readers using otoliths was lower in our study than the values reported for Longnose Gars from South Carolina estuaries (exact agreement = 63%, agreement within 1 year = 90%; Smylie et al. 2016). Although cleithra have never been used to age gars, the precision between readers using the cleithra of Spotted Gars was similar to those reported for Northern Pike from North Dakota (CV = 10%, exact agreement = 41%) and Wisconsin (CV = 11%, exact agreement = 49%; Faust et al. 2013). However, the precision between readers using the cleithra of Shortnose and Longnose gars was low and more comparable to that for White Suckers in Wyoming (CV = 16.2%; Quist et al. 2007). It is important that calcified structures be both accurate and precise, but in the absence of validation, assessments of precision between readers can determine the readability of a structure and help guide future validation studies. As we found minimal reader bias and relatively high agreement among readers using branchiostegal rays and pectoral fin rays, future accuracy assessments should focus on those structures.

The ability to produce a precise age estimate between readers was highly dependent on the age of the individual fish and the structure used. For example, both cleithra and otoliths resulted in poor precision between readers for

Spotted, Shortnose, and Longnose gars older than age 10. Readers had difficulty identifying annuli in these structures in older fish, likely due to the processing techniques used in our study. To our knowledge, cleithra have never been used to age gars, but they are commonly used to age other long-lived fishes, such as Northern Pike (Faust et al. 2013) and Muskellunge (Casselman 1979; cited by Casselman 1996). Cleithra were read whole under a dissection microscope (Faust et al. 2013), but because the bone had become opaque in older specimens the annuli were less distinct, apparently leading to the inconsistencies in our reader bias plots. Reducing the boiling increments (Redmond 1964; Quist et al. 2012) and sanding the structure may increase the repeatability of age estimates, especially for the thicker bones in older fish. The poor precision between readers using sagittal otolith sections may also be attributable to the preparation method used in our study. Although otoliths are the preferred structure for aging many species of fish (Maceina et al. 2007) and the only structure that has been validated for any species of gar (i.e., Alligator Gar; Buckmeier et al. 2012), difficulties identifying growth patterns in sectioned gar otoliths have been reported (Johnson and Noltie 1997; Ferrara 2001; Murie et al. 2009). Several preparation methods have been used to view annuli in gar otoliths (Ferrara 2001; Buckmeier et al. 2012; Smylie et al. 2016). We chose to set otoliths in epoxy resin and to make transverse cross sections (as in Smylie et al. 2016) in order to obtain sections of consistent thickness. Smylie et al. (2016) found strong

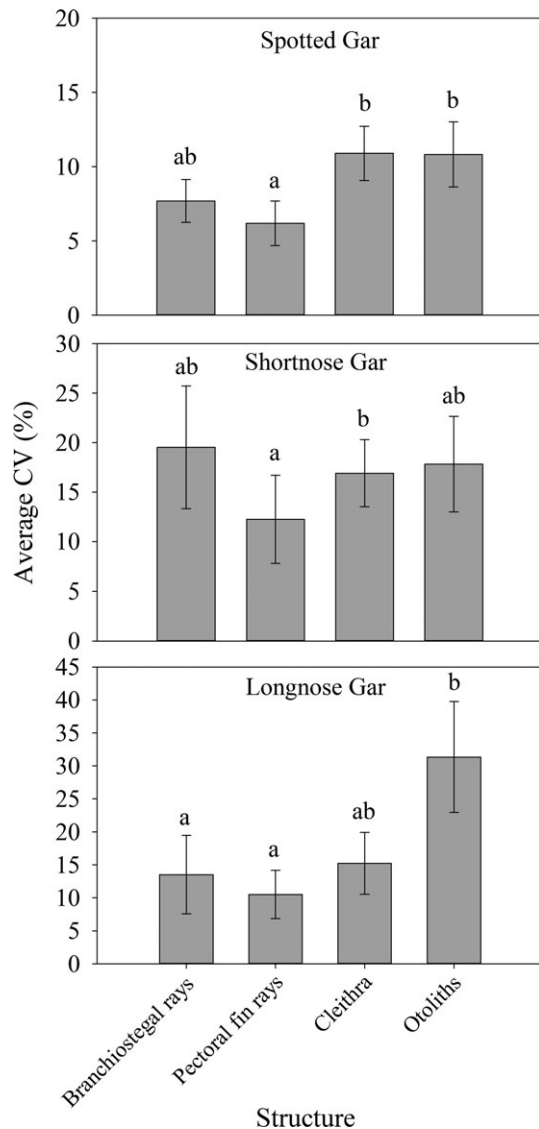


FIGURE 10. Average coefficients of variation between readers for age estimates from branchiostegal rays, pectoral fin rays, cleithra, and otoliths of Spotted, Shortnose, and Longnose gars. The error bars represent 95% confidence intervals around the average CVs. Different lowercase letters denote significant differences ($\alpha = 0.05$) according to a nonparametric Kruskal–Wallis test with a Tukey HSD post hoc test. Note that the scale of the y-axis varies by species.

agreement in the age estimates of Longnose Gars between readers using this technique, but no visible growth patterns were present in whole or sectioned Florida Gar *Lepisosteus platyrhincus* otoliths (Murie et al. 2009). In addition, Ferrara (2001) noted difficulty identifying annuli in sectioned otoliths for Alligator, Longnose, and Spotted gars and therefore used whole otoliths for age estimation (Ferrara 2001).

The poor precision between readers in our study may further be attributed to challenges inherent in otolith processing, in that otoliths must be mounted in epoxy at the

correct angle with a low margin of error; otherwise the annuli appear distorted in cross sections. In addition, gar otoliths grow in several different planes, which can cause the nucleus or outer annuli to become obscured when the plane of growth and the plane of sectioning differ (Ferrara 2001). These factors likely contributed to the inconsistencies in age estimates between readers in our study. When we were able to make successful transverse sections from whole otoliths, distinct annuli were identifiable in most of the section; therefore, we suggest that future studies pair our methods with sanding techniques (similar to those in Buckmeier et al. 2012) to view multiple planes in each otolith.

Although our data indicate that age estimates obtained using pectoral fin rays and branchiostegal rays were moderately precise between readers, we found inconsistencies in age estimates across structures. Age differences among calcified structures suggest that age estimates from certain structures are inaccurate and indicate the need for validation to determine which structure provides the most accurate ages of gars. In the absence of validation, readability can be used to coarsely evaluate age estimation techniques and identify the structure or structures most likely to be successfully validated. In this study (and using our preparation techniques), branchiostegal rays and pectoral fin rays were more readable than cleithra and otoliths for all age-classes. While the use of pectoral fin rays has the benefit of being nonlethal (Collins and Smith 1996), these rays provided the lowest mean and maximum age estimates of the four structures. Underestimation of age relative to the true age using pectoral fin rays has also been reported in other species (Rossiter et al. 1995; Whiteman et al. 2004). Based on their high readability, branchiostegal rays are the structure most commonly used to age gars (Redmond 1964; Murie et al. 2009), but age estimates using this structure have not been validated for any species. Although it is possible for a structure to produce precise age estimates among readers, those estimates may not necessarily represent the true age, further emphasizing the need for validation. The use of inaccurate age estimates to develop age-related metrics for management may lead to mismanagement of fish stocks in this ancient lineage of fish, which may be under increased harvest pressure from recreational anglers. Our study indicates that branchiostegal rays and pectoral fin rays can be prioritized for future validation studies to allow for the development of accurate age-related metrics for the management of gars.

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