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ARTICLE

Utility of Alligator Gar Age Estimates from Otoliths, Pectoral Fin Rays, and Scales

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Abstract
We evaluated annulus formation and readers' ability to recognize known annuli from otoliths, pectoral fin rays, and scales of alligator gar *Atractosteus spatula* using known-age (0 and 1 years) and chemically marked fish. Chemical marks were generally associated with annuli, and confirmed annulus formation occurred around May. In all bony structures, annuli appeared as narrow translucent lines that immediately preceded wider opaque bands; however, annuli in pectoral fin rays and scales were more variable in appearance than otoliths. In otoliths, the number of observed annuli corresponded with the number of years fish were at large for 96% of the fish. In contrast, periodicity of annuli in pectoral fin rays and scales only corresponded with the number of years fish were at large in 19% and 12% of the fish, respectively. Precision of age estimates was initially poor for all structures; percent agreement between two independent readers was only 49% for otoliths, 43% for pectoral fin rays, and 37% for scales. Agreement increased to 83% for otoliths, 65% for pectoral fin rays, and 60% for scales following a second independent age assessment. Final average coefficients of variation between readers were 2, 5, and 11% for otoliths, pectoral fin rays, and scales, respectively. Increased precision was the result of experience and an improved ability to identify annuli because of chemical reference marks. Although correct identification of annuli required substantial training, our study validates annulus formation in otoliths of alligator gar. In contrast, annulus formation was not validated for pectoral fin rays or scales from alligator gar older than age 6, but age estimates from pectoral fin rays of young alligator gar may have some utility.

Many populations of alligator gar *Atractosteus spatula* have declined (Jelks et al. 2008), and remaining populations support valuable sport and commercial fisheries. Lack of validated age estimation techniques (Beamish and McFarlane 1983) for this species hinders its conservation and management because accurate age data are needed to estimate growth, recruitment, and mortality, and to assess stock structure. Traditionally, the largest pair of branchiostegal rays have been used to estimate age of lepisosteids (Netsch and Witt 1962; Klaassen and Morgan 1974; Johnson and Noltie 1997; Love 2004; Murie et al. 2009; Sutton et al. 2009). However, this structure may not be useful for alligator gar because branchiostegal rays from large fish can be opaque and early annuli may have eroded (Ferrara 2001; Brinkman 2008). Instead, whole otoliths (Ferrara 2001; Brinkman 2008), otolith sections (DiBenedetto 2009), and sectioned scales (Brinkman 2008; DiBenedetto 2009) have been used to estimate age of alligator gar. While Brinkman (2008) and DiBenedetto (2009) reported that ages assigned to otoliths and scales of the same fish were somewhat correlated, accuracy of age estimation techniques has not been assessed for alligator gar or any lepisosteid, regardless of the structure or method used.

For most freshwater fish species in North America, otoliths provide age estimates with greater accuracy and precision than spines or scales (e.g., Erickson 1983; Heidinger and Clodfelter 1987; Hining et al. 2000; Buckmeier et al. 2002; Maceina and Sammons 2006). However, spines and scales continue to be used because they provide nonlethal methods of age estimation (Maceina et al. 2007). For depleted and economically valuable stocks of alligator gar, sacrificing fish may not be feasible. As

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a result, nonlethal age estimation methods are desirable, even if data are less reliable. Evaluations of alligator gar age estimation techniques are needed to assess the utility and limitations associated with various methods.

The fundamental assumption of fish age estimation from bony structures requires the correspondence of growth marks (i.e., annuli) with the number of years survived by each fish (Van Oosten 1928). Both known-age (e.g., Erickson 1983; Heidinger and Clodfelter 1987; Buckmeier et al. 2002) and chemically marked (e.g., Babaluk and Campbell 1987; Mantini et al. 1994; McFarlane and Beamish 1995; Hining et al. 2000) fish have been used to test this assumption. Use of known-age fish is preferred because this technique allows validation of absolute age, annulus formation, and a reader's ability to identify annuli (Van Oosten 1923; Campana 2001; Maceina et al. 2007). Chemically marked fish also allow validation of annulus formation and annulus identification by correlating the number of annuli past a chemical mark with the number of years since the fish was marked (Campana 2001). Both techniques require evaluation over a range of age-classes to ensure annuli are formed throughout the life of the fish (Beamish and McFarlane 1983; Campana 2001). We used a combination of known-age, chemically marked, and wild fish of unknown age to evaluate the utility and limitations of alligator gar age data derived from otoliths, pectoral fin rays, and scales. Specifically, we (1) determined if the number of observed annuli corresponded with the number of years at large, (2) quantified the ability of readers to identify known annuli, and (3) estimated the precision of age estimates between readers for each structure.

METHODS

Fish collection.—Known-age alligator gar were available for age-0 ($N = 6$; 337–452 mm total length [TL]) and age-1 ($N = 3$; 598–642 mm TL) fish. Known-age fish hatched 4 June 2010 at Heart of the Hills Fisheries Science Center (HOHFSC), Mountain Home, Texas. Fish were raised in aquaria through 19 d, transferred to outdoor tanks from 20 to 99 d, and finally stocked into a 0.2-ha research pond. Forage in outdoor tanks and the research pond included mosquitofish *Gambusia* spp., sunfish *Lepomis* spp., and common carp *Cyprinus carpio*. Fish were sacrificed at 90–118 d (age 0) and at 472 d (age 1).

Chemically marked alligator gar (1,295–2,032 mm TL; $N = 41$) were collected from Choke Canyon Reservoir and the Trinity River, Texas, in May 2009 and May 2010. After capture, these wild fish were injected in the dorsal musculature with oxytetracycline (OTC; Liquamyacin LA-200; Pfizer, New York, New York) to mark bony structures. Initially, fish received OTC at 100 mg/kg of body weight ($N = 6$; 0.50 mL/kg), but the dosage was reduced to 75 mg/kg body weight ($N = 35$; 0.38 mL/kg) to reduce the chance of mortality (Weber and Ridgway 1962; McFarlane and Beamish 1987). Following injection, OTC-marked fish were stocked into a 1.0-ha research pond at HOHFSC and sacrificed on 17 August 2011. Prior to harvest, three mortalities were recovered at 14, 105, and 565 d postinjection. Forage in the pond included largemouth bass *Micropterus salmoides*, sunfish, and common carp. Alligator gar were also fed Aquamax-Largemouth floating pellets (PMI Nutrition International, Brentwood, Missouri) to supplement their diet.

To increase sample sizes and age-classes for precision estimates of each structure, we also collected otoliths, pectoral fin rays, and scales from wild alligator gar (520–2,210 mm TL; $N = 47$) of unknown age. Unknown-age alligator gar were collected from Choke Canyon Reservoir, the Trinity River, and Trinity Bay, Texas, from April 2008 through August 2011.

Structure preparation.—Bony structures used for age estimation included the largest pair of otoliths (assumed to be sagittae, which are largest in nonostariophysean fish; Secor et al. 1991; Long and Stewart 2010), the anteriormost pectoral fin rays, and scales. Otoliths were collected from all alligator gar; after removal they were wiped clean, dried, and stored in vials. Pectoral fin rays and scales were collected from all alligator gar except the six age-0 fish and one OTC-marked fish that died 14 d postinjection. The anteriormost pectoral fin rays were clipped below the articulation, near the body using a side cutter (DeVries and Frie 1996). Unlike pectoral spines which are a single bone (e.g., in ictalurids), alligator gar pectoral fin rays comprised four to six small bones bound with connective tissue. These bones were not separated and were processed as a single structure. Collection sites for scales varied, but typically four to six lateral scales were collected, either from near the head or dorsal fin. Prior to storage, pectoral fin rays and scales were boiled in water and excess tissue was removed (Johnson and Noltie 1997; DiBenedetto 2009; Murie et al. 2009). With pectoral fin rays, caution was taken not to separate the individual bones during boiling. Pectoral fin rays and scales were dried and stored in coin envelopes.

Initial attempts to assign ages using whole otoliths (according to Ferrara 2001) and transverse otolith sections (similar to DiBenedetto 2009) were unsuccessful. Growth marks were visible in otolith sections; however, marks in older fish were difficult to separate near the ventral edge because the plane of calcium deposition appeared to have shifted. We chose to use ground otoliths because they allowed the viewing angle to be adjusted. Otoliths were ground in the transverse plane to the nucleus, which was located near the distal-anterior edge (Figure 1). Grinding was accomplished with a variable-speed rotary tool equipped with a grinding stone (number 85422; Dremel, Racine, Wisconsin). The rotary tool was held in a small vice, and otoliths were ground against the stone until the concave anterior portion of the otolith was flat and the nucleus was visible (Figure 1). Because the otoliths were asymmetrical, this only required a small portion of the otolith to be removed. While grinding, we used rubber-coated forceps to secure otoliths, and caution was taken to ensure the anterior-posterior axis of the otolith was held perpendicular to the stone. If otoliths were not ground perpendicular to the anterior-posterior axis, annuli near the ventral edge were difficult to identify. Following grinding,

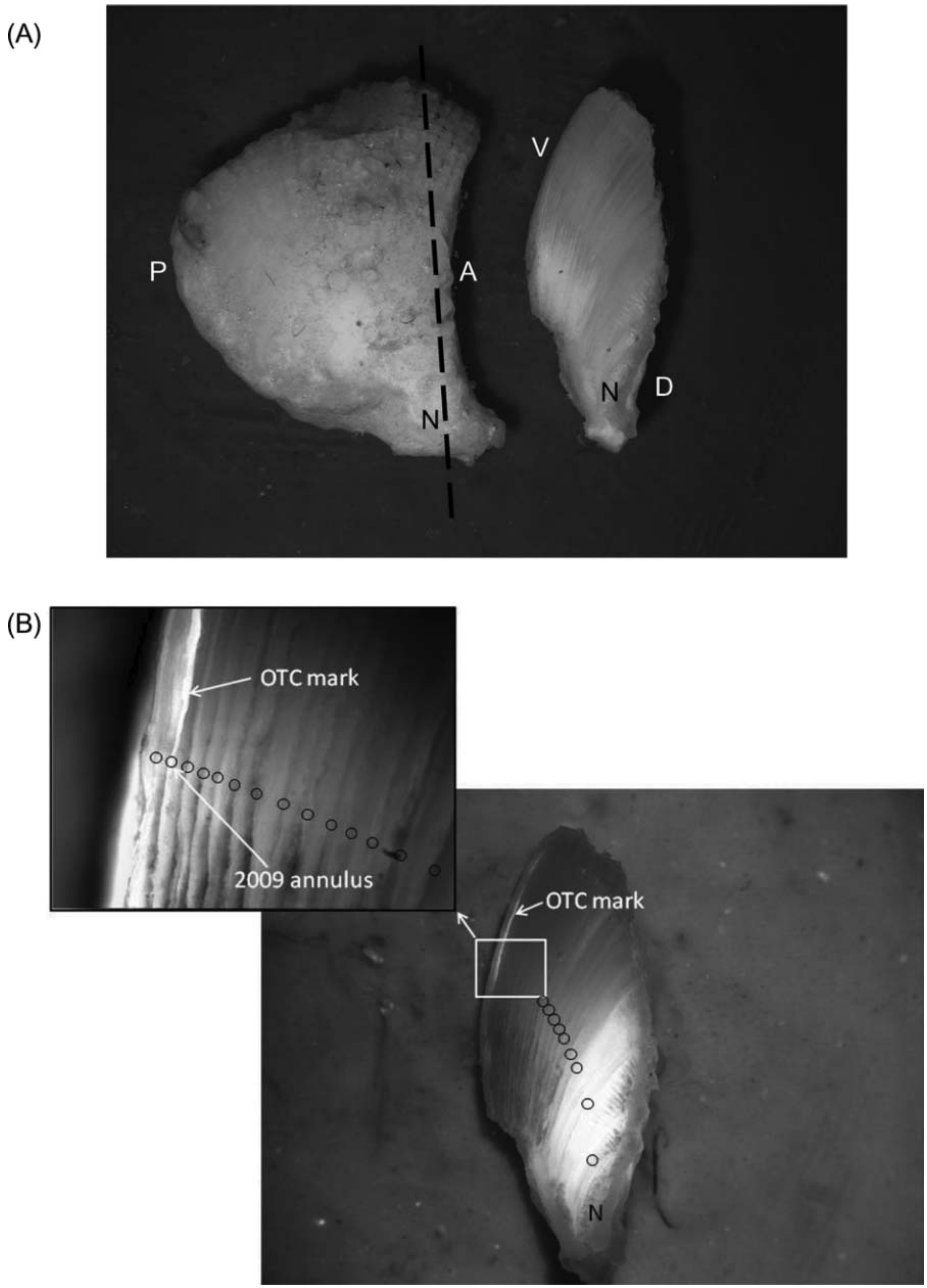

FIGURE 1. **(A)** Photograph of the whole left otolith and ground right otolith from a 1,825-mm alligator gar noting orientation (P = posterior, A = anterior, V = ventral, D = dorsal). To view annuli in the transverse plane, we held the posterior end of the otolith and ground the anterior portion to the dashed line. The nucleus (N) was located near the dorsal-anterior tip, and annuli formed along the ventral edge. **(B)** Photograph of the ground right otolith in (A) noting 23 annuli (o) and the OTC mark on the 2009 annulus, near the ventral edge. This fish was injected in May 2009 and died in November 2010.

otoliths were hand-polished using wetted 1,500-grit carborundum sandpaper.

Pectoral fin rays and scales were sectioned transversely using an IsoMet saw (Model 11-1280-16; Buehler, Lake Bluff, Illinois) equipped with a diamond-coated wafering blade. Structures were mounted in the vice of the saw and cut through the nucleus to yield two or three 0.35- to 0.40-mm thick sections. Each transverse section was mounted on a glass slide using Crystalbond (Buehler, Lake Bluff, Illinois). This process resulted in sections similar to those of Brinkman (2008) and DiBenedetto (2009).

Age estimation.—Polished otoliths were imbedded in a block of clay and submersed in water to reduce reflection. Otoliths were viewed with a dissecting microscope (7-115×) and illuminated with a 1-mm-diameter, single-strand fiber optic filament (Heidinger and Clodfelter 1987; Buckmeier et al. 2002). Separation of annuli near the ventral edge of otoliths with more than 10 annuli often required high magnification (≥75×) and changing the viewing angle of the otolith. Pectoral fin ray and scale sections were also submersed in water to reduce reflection and viewed with a dissecting microscope (7–115×) using transmitted light.

For each structure, age and corresponding year-class were estimated independently by two readers, each with at least 15 years of fish age estimation experience. Age assignments were made without reference to fish length, age (for known-age fish), or OTC location (for OTC-marked fish). To reduce bias, structures from known- and unknown-age fish were pooled. For pectoral fin rays and scales, each reader assigned an age after examining all sections (and bones of the pectoral fin ray). For fish that had been injected with OTC, age- and year-class were estimated prior to illuminating the OTC mark with ultraviolet light. Following age assignment, the OTC mark was illuminated and, if present, its location was identified in relation to the nearest annulus based on the assigned age. Because most fish (93%) were marked in May 2009 or May 2010, OTC marks were expected to overlap an annulus because annulus formation in lepisosteids is thought to occur in May–June (Netsch and Witt 1962; Ferrara 2001). Following initial age assignments by each reader, a second independent age assessment was conducted by the two readers for all structures for which readers were not in agreement. Structures which readers had assigned the same age during the first age assessment were not read a second time. After the second age assignment, any remaining disagreements were reconciled by the two readers viewing the structures together to derive a consensus age assignment and OTC location. Using this process, readers were able to assign consensus ages to all but one pectoral fin ray section, which was destroyed during processing.

Data analyses.—For each bony structure, we calculated the percent of alligator gar in which the number of annuli corresponded with the number of years at large based on consensus age assignments. For known-age fish, this required correct assignment of the year the fish hatched (i.e., 2010), whereas for OTC-marked fish it required identification of the correct location of the OTC mark (i.e., on the 2009 or 2010 annulus). The expected location of the OTC mark was based on the injection date, fish having zero to two annuli beyond the mark depending on their time at large (range = 14–830 d). Deviations from expected OTC locations indicated that annuli were not recognized, that annuli had not formed, that checks were counted as annuli, or that the fish failed to be marked with OTC. To identify structural biases, we plotted assigned ages from pectoral fin rays and scales against those from otoliths using all available fish (i.e., known-age, OTC-marked, and wild).

Precision of age estimates from each structure was evaluated using data from known-age, OTC-marked, and wild alligator gar. We calculated overall percent agreement between readers, the average coefficient of variation (CV = 100 × SD/mean; Chang 1982; Kimura and Lyons 1991; Campana et al. 1995), and constructed age bias graphs (similar to Campana et al. 1995). Percent agreement between readers and CV values were calculated following initial assignment of ages for each structure and again after the second age assessment. Agreement and CV values following the second reading reflected the overall agreement and variation (i.e., it included structures which readers were initially in agreement). Age bias graphs were used to assess bias associated with each reader. For each structure, assigned ages from reader 1 were plotted against assigned ages from reader 2 following both independent readings. If readings were unbiased, plots conformed around a line of equivalence (Campana et al. 1995).

Von Bertalanffy growth curves were used to determine if differences in consensus age assignments from each structure affected estimates of growth. Because fish were collected from various waters, these data did not reflect characteristics of any specific population. However, growth of alligator gar in Texas waters appears similar enough (Texas Parks and Wildlife Department [TPWD], unpublished data) to allow realistic inferences regarding the effects of age estimation differences between structures on growth estimates. Pairwise comparisons (between structures) of von Bertalanffy growth curves were made using likelihood ratio tests (Kimura 1980). Analyses were limited to fish where consensus age assignments were derived for all structures ($N = 89$).

RESULTS

Alligator gar in this study represented a broad range of age-classes, including young of the year, juvenile, and mature fish. Known-age alligator gar were age 0–1, OTC-marked fish were age 4–31, and wild fish were age 0–29 based on consensus age estimates from otoliths. In OTC-marked fish, injections successfully produced recognizable reference marks in all otoliths and most pectoral fin ray (93%) and scale (78%) sections. Oxytetracycline marks generally overlapped otolith annuli for all age-classes (Figures 1, 2) and in pectoral fin rays and scales from young fish, confirming annulus formation occurred around May.

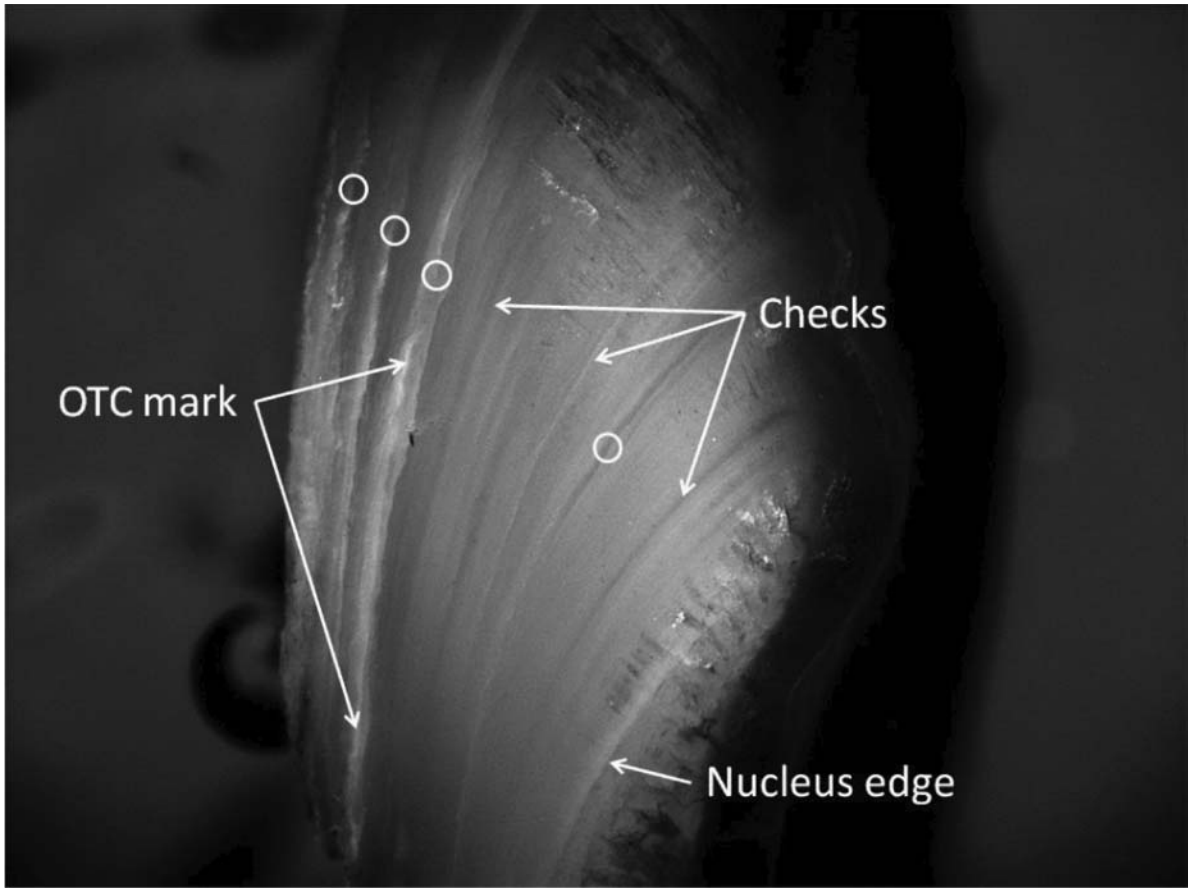

FIGURE 2. Photograph of the ground right otolith from a 1,325-mm alligator gar noting four annuli (o), growth checks, and the OTC mark on the 2009 annulus. This fish was injected in May 2009 and died in August 2011.

Clarity and location of annuli and corresponding OTC marks varied with fish age. In young fish, annuli were continuous and typically spanned much of the bony structure (Figure 2). In older fish, otolith annuli were often broken and limited to the ventral tip (Figure 1). In pectoral fin rays and scales from older fish, OTC marks, when present, were often limited to small areas of each structure. Frequently, OTC marks in pectoral fin rays and scales from older fish were not associated with visible annuli.

Observed annuli appeared as narrow translucent lines that immediately preceded wider opaque bands. In otoliths, these lines appeared as clear or white “fractures” when illuminated with the fiber optic filament from the side and across the polished surface (Figures 1, 2). Often, accessory marks and checks were also apparent in otoliths, especially between early annuli (Figure 2). Typically, these marks were less distinct and did not appear as a fracture when illuminated. The appearance of annuli in sections of pectoral fin rays and scales was more variable than in otoliths. These structures contained numerous accessory marks and checks throughout the sections, and annuli sometimes consisted of two or three closely spaced growth rings. In pectoral fin ray sections, annuli were observed in all of the bones, but ages were assigned based on the two largest bones, which typically contained the most annuli.

In otoliths, the number of observed annuli corresponded with the number of years fish were at large, validating annulus formation. Based on consensus age assignments, readers correctly estimated ages for all known-age fish using otoliths. For OTC-marked fish, readers missed a single annulus in two of the otoliths. Thus, readers correctly identified all known annuli in 96% of otoliths ($N = 50$) from known-age and OTC-marked alligator gar.

In contrast with otoliths, periodicity of annuli observed in pectoral fin ray and scale sections (from fish $\geq$ age 6) did not correspond with the number of years at large. Oxytetracycline marks were often past the last identifiable annulus or missing altogether, indicating that annuli did not form every year in these structures. As a result, readers tended to underestimate fish age. Based on consensus age assignments for OTC-marked fish, readers missed one annulus in 48% of the pectoral fin rays and 33% of the scales, and at least two annuli (or the OTC mark was missing) in 40% of the pectoral fin rays and 58% of the scales. Only one OTC-marked fish in the sample was $<$ age 6 (an age-4 fish). For this fish, the number of annuli observed in pectoral fin ray and scale sections corresponded with the number of years at large. After pooling data for known-age and OTC-marked fish, readers only identified all known annuli for 19% of the pectoral fin rays ($N = 43$) and 12% of the scales ($N = 43$).

Graphical plots of consensus age assignments from pectoral fin rays and scales versus otoliths also indicated that readers underestimated ages of older fish, relative to otoliths (Figure 3). In general, both structures produced truncated age distributions with maximum age estimates of 23 years for pectoral fin rays and 20 years for scales compared with 31 years for otoliths. Otoliths and pectoral fin rays produced similar age estimates in fish $\leq$ age 6, whereas otolith and scales were similar through age 4 (Figure 3). Assigned ages, based on scales, were also more variable than estimates from pectoral fin rays. For example, 18 age-9 fish (based on otoliths) were assigned to seven different age-classes using scales (age 4–10).

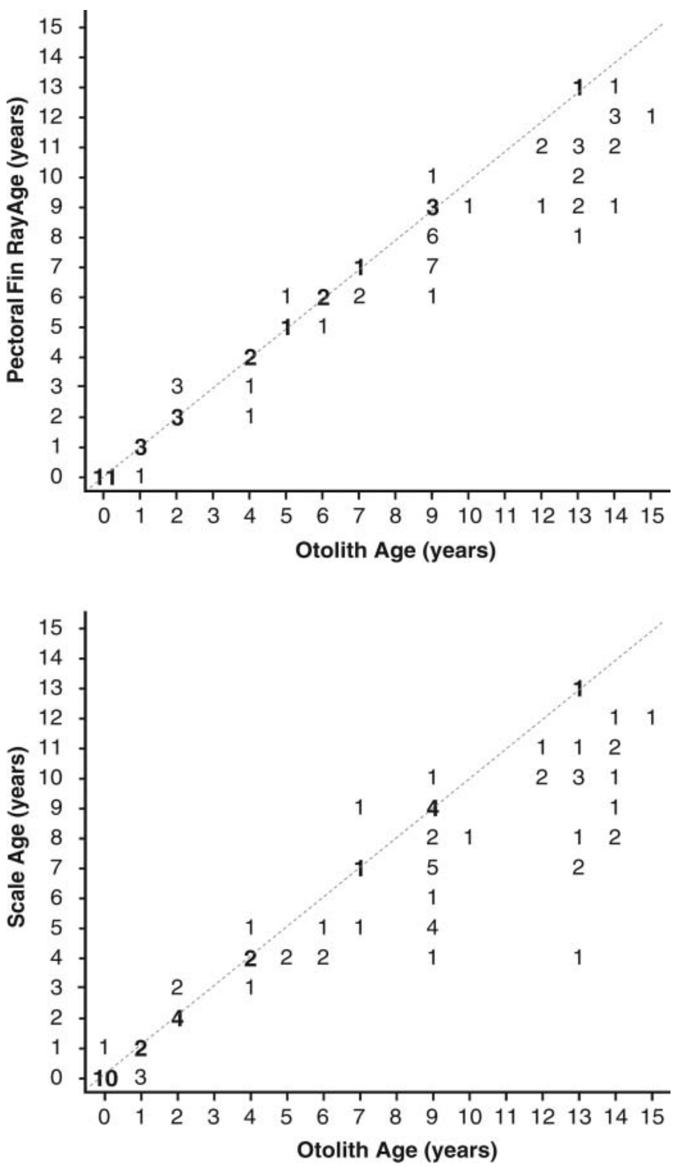

FIGURE 3. Comparison of consensus age estimates from pectoral fin rays versus otoliths (top) and scales versus otoliths (bottom). Numbers indicate sample size. Bold values represent 1:1 equivalence (dashed line) and correspond to the number of fish where age estimates from the two structures agreed.

While readers were better able to identify known annuli in otoliths relative to pectoral fin rays and scales, precision of assigned ages was initially poor for all structures. Following the first attempt to assign ages, percent agreement between the two independent readers was only 49% for otoliths, 43% for pectoral fin rays, and 37% for scales. Similarly, CV values were 12% for otoliths, 10% for pectoral fin rays, and 15% for scales. After a second age assessment for structures about which readers initially disagreed, overall precision increased by 22–34% for each structure. Ultimately, readers were able to agree on 83% of the otoliths, were within 1 year for 95%, and had an average CV of 2%. For pectoral fin rays, reader agreement was 65%, 91% of the age assignments were within 1 year, and average CV was 5%. After two attempts, readers only agreed on 60% of the scales, were within 1 year for 71%, and had an average CV of 11%.

No reader bias was evident for any of the structures after the second age assessment (Figure 4). The only bias that was initially evident was for otoliths by reader 1. After the first attempt to assign age, reader 1 tended to have higher age estimates from otoliths than reader 2 for fish > age 20 (Figure 4). While no other bias was evident, variability in age estimates between readers was greater for pectoral fin rays and scales as compared with otoliths, even after the second assessment.

Fitted von Bertalanffy growth curves from each structure suggested different patterns of growth, especially in older fish (Figure 5). Pairwise comparisons between structures revealed that growth estimated from otoliths differed with that from scales ($P < 0.001$) and pectoral fin rays ($P = 0.042$), while estimates between scales and pectoral fin rays was similar ($P = 0.198$). By age 5, divergence in growth estimates occurred; mean length-at-age estimates were greater for scales (1,255 mm) and pectoral fin rays (1,180 mm) than for otoliths (1,139 mm). Asymptotic length was estimated to be 2,021 mm from scales, 2,132 mm from pectoral fin rays, and 1,962 mm from otoliths.

DISCUSSION

Use of known-age and OTC-marked fish allowed us to test the assumption that growth marks in otoliths, pectoral fin rays, and scales of alligator gar corresponded with the number of years at large. For otoliths, we found this assumption to be valid, although annulus formation changed with fish age. Unlike early annuli that spanned much of the otolith and were continuous, outermost annuli were limited to the ventral edge and were often intermittent. McFarlane and Beamish (1995) previously noted similar findings for sablefish *Anoplopoma fimbria* and proposed that growth becomes limited to the ventral otolith surface for many long-lived species.

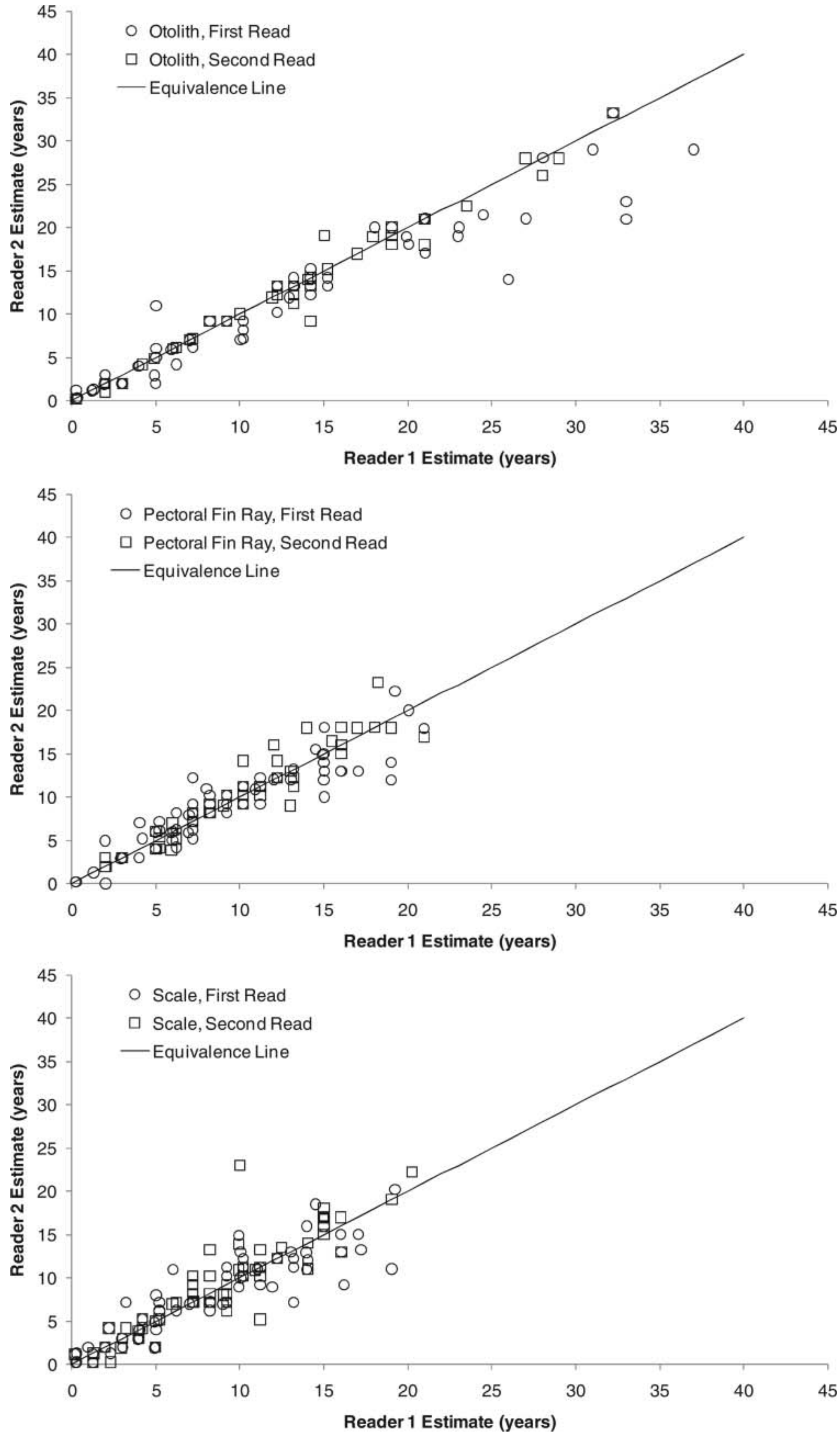

FIGURE 4. Age bias graphs comparing initial (circles) and second (squares; for those initially disagreed on) age estimates between readers for otoliths (top), pectoral fin rays (middle), and scales (bottom). The 1:1 equivalence is represented by the solid line.

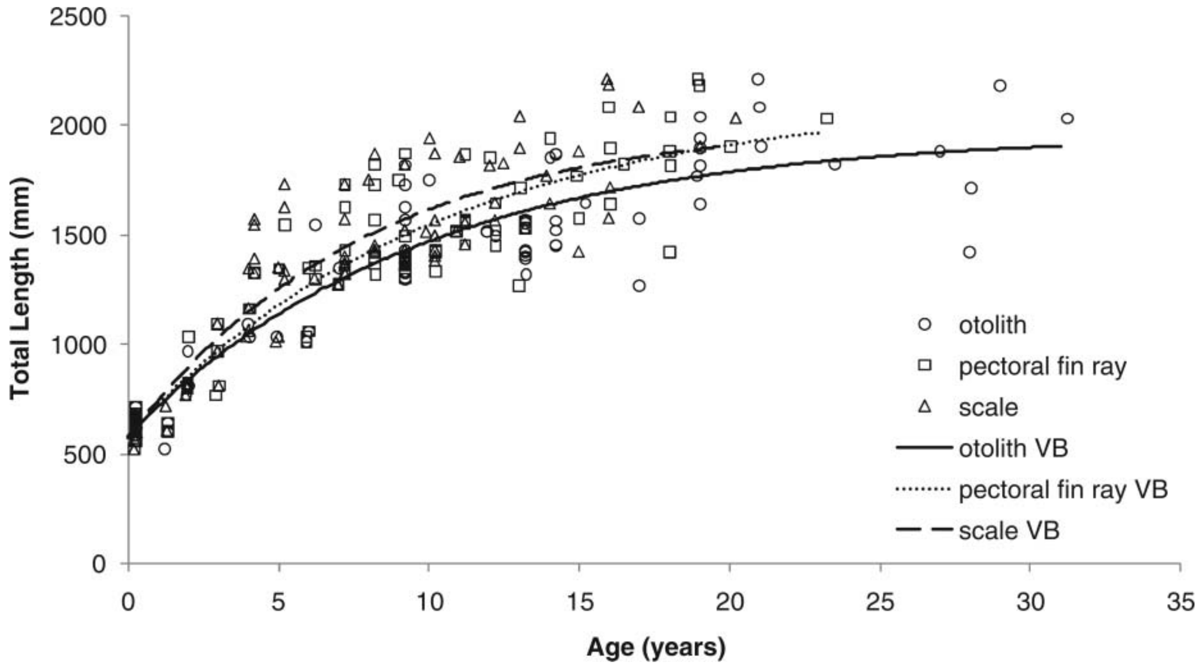

FIGURE 5. Age–length data based on consensus age estimates from otoliths (circles), pectoral fin rays (squares), and scales (triangles). Lines represent associated von Bertalanffy (VB) growth curves for otoliths (solid), pectoral fin rays (dotted), and scales (dashed).

Validation of annulus formation in otoliths of alligator gar suggests that age estimates from ground otoliths can be used to estimate population metrics and assay stock structure. However, difficulties associated with annulus identification warrant some caution. It was more difficult to estimate ages from alligator gar otoliths than from otoliths of other freshwater fish species (e.g., crappie *Pomoxis* spp., largemouth bass, and flathead catfish *Pylodictis olivaris*). Identification of annuli was particularly problematic in otoliths of fish older than 10–20 years. In these fish, annulus identification often required high magnification and changing the viewing angle. Not surprisingly, these challenges coincided with reduced growth as fish approached their asymptotic length based on the von Bertalanffy growth model. Difficulty identifying annuli near the ventral edge may ultimately lead to underestimates of true age, especially as alligator gar near their maximum age (>60 years; TPWD, unpublished data).

Other difficulties included identification of the first annulus and the presence of checks and accessory marks between early annuli. While many of these marks could be eliminated because they were less distinct or did not appear as fractures, some marks appeared very similar to annuli. Because our sample lacked OTC-marked fish less than age 4, we were unable to determine for certain if these were annuli or checks. Fish known to be age 0 and age 1 did confirm that readers were able to identify the first annulus, but further research is needed to identify the characteristics of early annuli and checks in young fish.

In contrast to otoliths, annulus formation was not validated for pectoral fin rays and scales from fish age 6 or greater. Known annuli and OTC marks were often missing from these structures, and, when present, OTC marks were limited to small areas of the pectoral fin ray and scale sections (often beyond identifiable annuli). Lack of annulus formation in scales has previously been suspected for other species. Hining et al. (2000) found that the proportion of scales of rainbow trout *Oncorhynchus mykiss* with OTC marks declined with fish age, and OTC marks were completely absent in age-3 fish.

Pectoral fin rays and scales did not provide age estimates of sufficient accuracy to estimate population metrics. We found that errors associated with both nonlethal methods resulted in underestimates of maximum age, overestimates of growth, and a smoothing of age-classes. This result was not surprising because estimates from spines, fin rays, and scales frequently underestimate age relative to otoliths for other species (e.g., Erickson 1983; Buckmeier et al. 2002; Maceina and Sammons 2006). Annuli in these structures were also difficult to separate near the outer edge. This difficulty has previously been noted in dorsal spines of walleyes *Sander vitreus* (Kocovsky and Carline 2000) and pectoral spines of flathead catfish (Nash and Irwin 1999). Other challenges included variability in the appearance of annuli and the presence of many accessory marks and checks throughout the structures. Because such errors can cause serious mismanagement of stocks (Beamish and McFarlane 1983; Beamish and McFarlane 1995), we discourage the use of pectoral fin rays or scales for estimating population metrics of alligator gar.

For young alligator gar, pectoral fin rays may provide some useful data. Assigned ages from pectoral fin rays were more accurate and precise than those from scales. Pectoral fin rays were also easier to remove, and removal was less invasive and less likely to cause infection. Though our sample size was admittedly small, we found that consensus age assignments from pectoral fin ray sections typically agreed with those from otoliths through age 6. Growth data were somewhat overestimated using pectoral fin rays but may be within acceptable bounds for some objectives (at age 5, predicted mean TL was within 41 mm of the estimate based on otoliths). Precision of age estimates from pectoral fin ray sections through age 6 was also good. If biologists choose to use pectoral fin rays for age estimation of alligator gar, we recommend that use be limited to fish ≤ 1,200-mm TL (this length corresponded with alligator gar ≤ age 6). Also, biologists should consider that growth variability may affect the utility of age data from pectoral fin rays, and that a length cap of 1,200 mm may result in upper age-classes being truncated.

The use of two independent age assessments followed by readers viewing remaining structures together to reconcile discrepancies produced accurate and precise age data from otoliths in this study. Although this method was somewhat nontraditional and required more effort than a single independent assessment, we found it useful because of the previously mentioned difficulties in identifying annuli. With greater experience, a second age assessment may not be needed, but we recommend this approach if agreement is poor following a first estimate. Also, we strongly encourage the use of chemically marked fish to train readers to identify annuli. Early bias observed for reader 1 was eliminated in a second age assessment. We attribute this correction and concurrent increases in precision to the confidence in annulus interpretation provided by the OTC marks. Association between OTC marks and annuli allowed readers to verify correct identification of annuli.

Our evaluation of annulus formation and assessment of the ability of readers to identify known annuli in otoliths, pectoral fin rays, and scales of alligator gar found that otoliths produced the most accurate and precise age estimates. However, recognition of annuli required substantial experience and training. Biologists using otoliths to assign ages to alligator gar should consider age data as estimated values and consider implications of age estimation errors. While pectoral fin ray sections appear to have some utility for estimating ages of young alligator gar, further assessment is necessary to adequately quantify their efficacy.

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REFERENCES

Babaluk, J. A., and J. S. Campbell. 1987. Preliminary results of tetracycline labelling for validating annual growth increments in opercula of walleyes. North American Journal of Fisheries Management 7:138–141.

Beamish, R. J., and G. A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society 112:735–743.

Beamish, R. J., and G. A. McFarlane. 1995. A discussion of the importance of aging errors, and an application to walleye pollock: the world's largest fishery. Pages 545–565 *in* D. H. Secor, J. M. Dean, and S. E. Campana, editors. Recent developments in fish otolith research. University of South Carolina Press, Columbia.

Brinkman, E. L. 2008. Contributions to the life history of alligator gar, *Atractosteus spatula*, in Oklahoma. Master's thesis. Oklahoma State University, Stillwater.

Buckmeier, D. L., E. R. Irwin, R. K. Betsill, and J. A. Prentice. 2002. Validity of otoliths and pectoral spines for estimating ages of channel catfish. North American Journal of Fisheries Management 22:934–942.

Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59:197–242.

Campana, S. E., M. C. Annand, and J. I. McMillan. 1995. Graphical and statistical methods for determining the consistency of age determinations. Transactions of the American Fisheries Society 124:131–138.

Chang, W. Y. B. 1982. A statistical method for evaluating the reproducibility of age determination. Canadian Journal of Fisheries and Aquatic Sciences 39:1208–1210.

DeVries, D. R., and R. V. Frie. 1996. Determination of age and growth. Pages 483–512 *in* B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

DiBenedetto, K. C. 2009. Life history characteristics of alligator gar, *Atractosteus spatula*, in the Bayou Dularge area of southcentral Louisiana. Master's thesis. Louisiana State University, Baton Rouge.

Erickson, C. M. 1983. Age determination of Manitoban walleyes using otoliths, dorsal spines, and scales. North American Journal of Fisheries Management 3:176–181.

Ferrara, A. M. 2001. Life-history strategy of Lepisosteidae: implications for the conservation and management of alligator gar. Doctoral dissertation. Auburn University, Auburn, Alabama.

Heidinger, R. C., and K. Clodfelter. 1987. Validity of the otolith for determining age and growth of walleye, striped bass, and smallmouth bass in power plant cooling ponds. Pages 241–251 *in* R. C. Summerfelt and G. E. Hall, editors. The age and growth of fish. Iowa State University Press, Ames.

Hining, K. J., J. L. West, M. A. Kulp, and A. D. Neubauer. 2000. Validation of scales and otoliths for estimating age of rainbow trout from southern Appalachian streams. North American Journal of Fisheries Management 20:978–985.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Díaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren Jr. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372–407.

Johnson, B. L., and D. B. Noltie. 1997. Demography, growth, and reproductive allocation in stream-spawning longnose gar. Transactions of the American Fisheries Society 126:438–466.

Kimura, D. K. 1980. Likelihood methods for the von Bertalanffy growth curve. U.S. National Marine Fisheries Service Fishery Bulletin 77:765–776.

Kimura, D. K., and J. J. Lyons. 1991. Between-reader bias and variability in the age-determination process. U.S. National Marine Fisheries Service Fishery Bulletin 89:53–60.

Klaassen, H. E., and K. L. Morgan. 1974. Age and growth of longnose gar in Tuttle Creek Reservoir, Kansas. Transactions of the American Fisheries Society 103:402–405.

Kocovsky, P. M., and R. F. Carline. 2000. A comparison of methods for estimating ages of unexploited walleyes. North American Journal of Fisheries Management 20:1044–1048.

Long, J. M., and D. R. Stewart. 2010. Verification of otolith identity used by fisheries scientists for aging channel catfish. Transactions of the American Fisheries Society 139:1775–1779.

Love, J. W. 2004. Age, growth, and reproduction of spotted gar, *Lepisosteus oculatus* (Lepisosteidae), from the Lake Pontchartrain Estuary, Louisiana. Southwestern Naturalist 49:18–23.

Maceina, M. J., J. Boxrucker, D. L. Buckmeier, R. S. Gangl, D. O. Lucchesi, D. A. Isermann, J. R. Jackson, and P. J. Martinez. 2007. Current status and review of freshwater fish aging procedures used by state and provincial fisheries agencies with recommendations for future directions. Fisheries 32:329–340.

Maceina, M. J., and S. M. Sammons. 2006. An evaluation of different structures to age freshwater fish from a northeastern US river. Fisheries Management and Ecology 13:237–242.

Mantini, L., M. V. Hoyer, J. V. Shireman, and D. E. Canfield Jr. 1994. Annulus validation, time of formation, and mean length at age of three sunfish species in north central Florida. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 46(1992):357–367.

McFarlane, G. A., and R. J. Beamish. 1987. Selection of dosages of oxytetracycline for age validation studies. Canadian Journal of Fisheries and Aquatic Sciences 44:905–909.

McFarlane, G. A., and R. J. Beamish. 1995. Validation of the otolith cross-section method of age determination for sablefish (*Anoplopoma fimbria*) using oxytetracycline. Pages 319–329 *in* D. H. Secor, J. M. Dean, and S. E. Campana, editors. Recent developments in fish otolith research. University of South Carolina Press, Columbia.

Murie, D. J., D. C. Parkyn, L. G. Nico, J. J. Herod, and W. F. Loftus. 2009. Age, differential growth and mortality rates in unexploited populations of Florida gar, an apex predator in the Florida Everglades. Fisheries Management and Ecology 16:315–322.

Nash, M. K., and E. R. Irwin. 1999. Use of otoliths versus pectoral spines for aging adult flathead catfish. Pages 309–316 *in* E. R. Irwin, W. A. Hubert, C. F. Rabeni, H. L. Schramm Jr., and T. Coon, editors. Catfish 2000: proceedings of the international ictalurid symposium. American Fisheries Society, Symposium 24, Bethesda, Maryland.

Netsch, N. F., and A. Witt Jr. 1962. Contributions to the life history of the longnose gar (*Lepisosteus osseus*) in Missouri. Transactions of the American Fisheries Society 91:251–262.

Secor, D. H., J. M. Dean, and E. H. Laban. 1991. Otolith removal and preparation for microstructural examination: a user's manual. The Belle W. Baruch Institute for Marine Biology and Coastal Research, Technical Publication 1991-01, University of South Carolina, Columbia. Available: www.cbl.umces.edu/∼secor/otolith-manual.html. (March 2012).

Sutton, T. M., A. C. Grier, and L. D. Frankland. 2009. Stock structure and dynamics of longnose gar and shortnose gar in the Wabash River, Indiana–Illinois. Journal of Freshwater Ecology 24:657–666.

Van Oosten, J. 1923. The whitefishes (*Coregonus clupeaformis*): a study of the scales of whitefishes of known ages. Zoologica (New York) 2:380–412.

Van Oosten, J. 1928. Life history of the lake herring (*Leucichthys artedi* Le Sueur) of Lake Huron as revealed by its scales, with a critique of the scale method. U.S. Bureau of Fisheries Bulletin 44:265–428.

Weber, D. D., and G. J. Ridgway. 1962. The deposition of tetracycline drugs in bones and scales of fish and its possible use for marking. Progressive Fish-Culturist 24:150–155.