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Are Age Estimates for Longnose Gar and Spotted Gar Accurate? An Evaluation of Sagittal Otoliths, Pectoral Fin Rays, and Branchiostegal Rays

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Abstract

We evaluated the accuracy and precision of age estimates from ground sagittal otoliths, sectioned pectoral fin rays, and whole branchiostegal rays of Longnose Gar *Lepisosteus osseus* and Spotted Gar *Lepisosteus oculatus* using fish marked with oxytetracycline (OTC). The presence of OTC time stamps in these calcified structures and the ability to correctly identify post–time stamp annuli varied greatly. We identified time stamps in 66.7% and 91.7% of the otoliths and 43.8% and 61.4% of the pectoral fin rays for Longnose Gars and Spotted Gars, respectively; OTC marks were not observed in branchiostegal rays. Annual increment periodicity was validated in ground sagittal otoliths through age 10 for both species. For fish older than age 10, accuracy declined to about 60%, with most estimates being less than the number of post–time stamp annuli by 1 year. Age estimates derived from pectoral fin rays were consistently less than the number of post–time stamp annuli because OTC marks were generally associated with the outer edge of this bone. Overall accuracy from pectoral fin rays was only 14.3% for Longnose Gars and 22.6% for Spotted Gars. Although the lack of time stamps in branchiostegal rays prevented formal evaluation, age estimates derived from this structure were substantially lower than those from otoliths. Precision among readers was poor for all structures, reflecting the difficulties associated with age estimation for these two species. Based on our evaluation, past age estimates from branchiostegal rays and pectoral fin rays should be considered suspect. Ages derived from sagittal otoliths can be reliable through age 10. However, we recommend that readers verify their ability to produce accurate estimates and consider the implications of underestimating age for fish greater than age 10. Future efforts should work to refine or develop alternative preparation procedures for otoliths to improve the visibility of annuli and validate age estimates for older age-classes.

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Ages estimated from calcified structures have been used to assess the life history, ecology, and population dynamics of Longnose Gar *Lepisosteus osseus* and Spotted Gar *L. oculatus* for more than half a century (Netsch and Witt 1962; Klaassen and Morgan 1974; Johnson and Noltie 1997; Ferrara 2001; Love 2004; Sutton et al. 2009; McGrath et al. 2016; Smylie et al. 2016). Historically, most age data for these species were derived from branchiostegal rays because this bone tends to have obvious growth increments that can be consistently interpreted by readers (Netsch and Witt 1962; Klaassen and Morgan 1974; Johnson and Noltie 1997; Love 2004; Sutton et al. 2009; McGrath et al. 2016). In recent years, pectoral fin rays and sagittal otoliths have also been used to estimate age. Sectioned pectoral fin rays were selected as a nonlethal alternative to branchiostegal rays by Glass et al. (2011). Sagittal otoliths were examined because branchiostegal rays from large individuals can be completely opaque and because of concerns that bones may underestimate the true age (Ferrara 2001; Smylie et al. 2016).

The fundamental assumption of fish age estimation from calcified structures is that the number of annuli corresponds to the number of years a fish has survived (Van Oosten 1929). Validation of age estimation techniques is critical because age estimation errors can lead to inaccurate estimates of vital rates, which can result in the mismanagement of stocks (Beamish and McFarlane 1983). To date, no age estimation technique has been validated for either Longnose Gar or Spotted Gar, creating uncertainty about the conclusions drawn from past studies. The findings of Buckmeier et al. (2012) provide further concern about the validity of age estimates derived from the bones of gar species. Those researchers reported that annual increment periodicity in sectioned pectoral fin rays and ganoid scales could not be confirmed for Alligator Gar *Atractosteus spatula* more than 6 years of age. In contrast, Buckmeier et al. (2012) were able to validate annual periodicity in ground sagittal otoliths from ages 0 through 31 in Alligator Gar.

Age estimation methods can be validated using fish of known age or fish that have time stamps in their calcified structures (e.g., fish that have been marked with oxytetracycline [OTC]). While known-age fish are preferred because both the true age and annual increment periodicity can be validated, the use of fish with time stamps is often more feasible (e.g., Babaluk and Campbell 1987; Casselman 1987; Mantini et al. 1992; McFarlane and Beamish 1995; Hining et al. 2000; Buckmeier et al. 2012). When time stamps are used, annual increment periodicity is confirmed if the number of putative annuli past the time stamp coincides with the number of years that have elapsed since the fish was marked (Casselman 1987; Campana 2001). To confirm that annuli form throughout life, validation studies must evaluate annual increment periodicity for all age-classes (Beamish and McFarlane 1983; Campana 2001). In this study, we used fish marked with OTC to evaluate the accuracy and precision of age estimates of Longnose Gar and Spotted Gar derived from ground sagittal otoliths, sectioned pectoral fin rays, and whole branchiostegal rays. Specifically, we (1) determined whether the number of post–time stamp annuli corresponded to the number of years since fish were injected across a broad age range of fish and (2) quantified the precision of age estimates between two readers for each structure.

METHODS

Fish collection and OTC time stamps.— The Longnose Gar (631–1,084 mm TL; n = 48) and Spotted Gar (429–866 mm TL; n = 88) used in this study were collected from O. H. Ivie, Buchanan, and Choke Canyon Reservoirs, Texas, from March 2009 through November 2010. Following capture, fish were transported to Heart of the Hills Fisheries Science Center near Mountain Home, Texas, where they were later implanted with passive integrated transponder tags for individual identification and injected with OTC (Liquamyacin LA-200; Pfizer, New York) to produce a time stamp in calcified structures. Immediately prior to injection, fish were weighed and measured (TL; mm). At the outset, we injected Spotted Gars with OTC at 100 mg/kg of body weight (0.50 mL/kg), but the dosage was reduced to 75 mg/kg (0.38 mL/kg) because the higher dosage resulted in some initial mortality (Weber and Ridgway 1962; McFarlane and Beamish 1987). At the conclusion of the study, 24 of the Spotted Gars had received the higher dosage; the remaining Spotted Gars and all Longnose Gars were injected at 75 mg/kg. Following injection, fish were stocked into outdoor research ponds. In addition, 11 Spotted Gars (347–450 mm TL) resulting from reproduction in the ponds were collected in November 2010 and subsequently injected with OTC (75 mg/kg) and returned to the ponds. These fish had hatched in either 2009 or 2010. All injections occurred between November and May, in hopes that the time stamps would be associated with annuli, which were believed to be completed between April and June based on observations by Ferrara (2001), Murie et al. (2009), Buckmeier et al. (2012), and Smylie et al. (2016). A subsample of fish were sacrificed in November 2010, and the remainder were sacrificed in August 2011. Upon sacrifice, all fish were again weighed and measured. Fish were held in the ponds from 182 to 828 d, resulting in 0–2 expected post–time stamp annuli. To provide seminatural conditions, ponds were stocked with natural forage, including centrarchids (e.g., Largemouth Bass *Micropterus salmoides* and Bluegill *Lepomis macrochirus*), Common Carp *Cyprinus carpio*, and Gizzard Shad *Dorosoma cepedianum*.

Structure preparation.— The calcified structures used for age estimation included sagittal otoliths, the anterior-most pectoral fin ray, and the largest branchiostegal ray. Otoliths were removed, cleaned, and stored using methods similar to those reported in Buckmeier et al. (2012) for Alligator Gar. Pectoral fin rays were cut next to the body and dried in coin envelopes. After drying, they were soaked in hot water to remove extra tissue and dried again before sectioning. During this process, care was taken not to separate the multiple individual bones that made up the anterior-most ray. Branchiostegal rays were removed and cleaned by gently boiling them in water to remove excess tissue, as in Johnson and Noltie (1997), and then dried in coin envelopes. All structures were stored in the dark to avoid deterioration of the OTC time stamps until they were processed for age estimation (Chilton and Beamish 1982).

Sagittal otoliths were prepared and viewed with methods similar to those validated for Alligator Gar (Buckmeier et al. 2012). Otoliths were ground in the transverse plane using a variable-speed rotary tool equipped with a grinding stone and then hand-sanded with 600- and 1,500-grit wetted sandpaper until the core area was clear. We prepared the otoliths so that when finished the ground surface was perpendicular to the anterior–posterior axis (Figure 1) because slight deviations from this angle resulted in structures that were difficult to interpret. Prepared otoliths were immersed in water and viewed with a variable-power stereomicroscope in the transverse plane and were illuminated with a fiber-optic filament. Initially, a microscope capable of magnification up to 45× was used (hereafter referred to as low magnification). However, after it was apparent that annuli near the ventral edge of the otoliths could not always be separated at this magnification, all otoliths were re-examined with a stereomicroscope capable of 115× magnification (hereafter referred to as high magnification).

Pectoral fin rays were prepared and viewed with methods similar to those described for Spotted Gars by Glass et al. (2011) and for Alligator Gars by Buckmeier et al. (2012). Pectoral fin rays were sectioned transversely near the proximal end where the structure was cut near the body. Four to five sections were acquired using a low-speed saw with a diamond-coated wafering blade. Sections were then mounted to glass slides using Crystalbond (Buehler, Lake Bluff, Illinois) and polished to <0.5-mm thickness. Pectoral fin ray sections were examined with a compound microscope at 100× and 200× magnification and illuminated with transmitted light (Figure 1).

Dry branchiostegal rays were examined whole with a stereomicroscope at 4–40× magnification depending on the size of the structure. A fiber-optic filament was used to transmit light through the branchiostegal ray at various angles, including from underneath and along the sides, to illuminate annuli (Figure 1). We found that putative annuli were more visible using this technique than using the methods reported by Murie et al. (2009), by which they were immersed in water and illuminated with transmitted light from below.

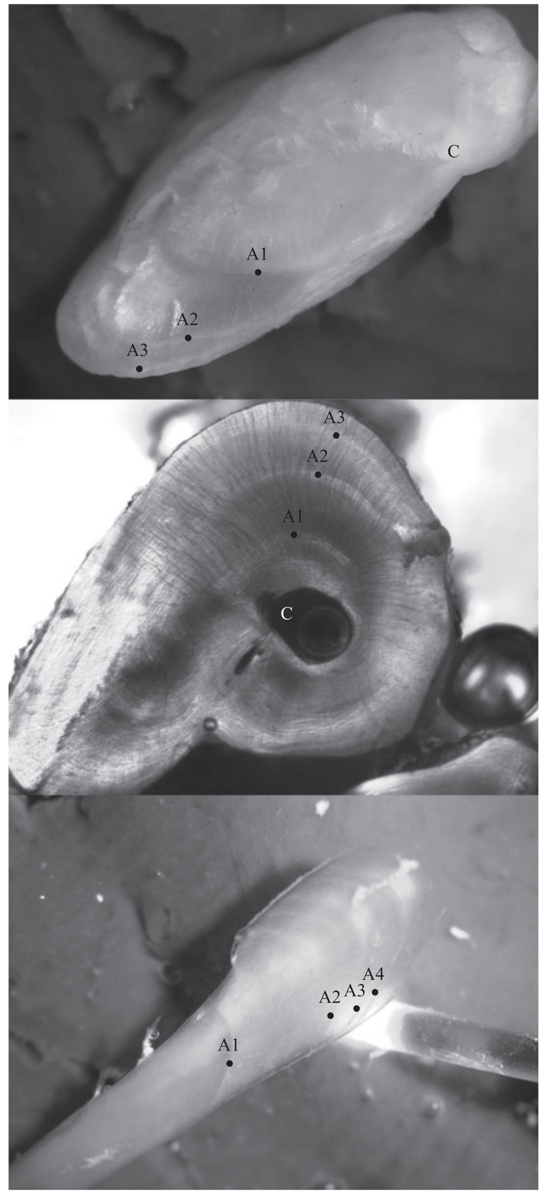

FIGURE 1. Images of a processed sagittal otolith (upper panel), a pectoral fin ray (middle panel), and a branchiostegal ray (lower panel) from a 616-mm Spotted Gar. The locations of putative annuli (A; black dots) and the core (C) are noted. The fish was estimated to be age 3 from the otolith and pectoral fin ray and age 4 from the branchiostegal ray.

Age estimation.— The appearance of putative annual increments and annuli differed among calcified structures (Figure 1). In ground sagittal otoliths and whole branchiostegal rays, annual increments included a relatively wide translucent zone that generally decreased in size toward the outer margin as well as a consistently narrow opaque zone. In otoliths, annuli were defined as the distal edge of the opaque zones because these margins appear as very bright fractures when illuminated with intense light from fiber-optic filament (Williams and Bedford 1974; Heidinger and Clodfelter 1987; Buckmeier et al. 2002, 2012). In whole branchiostegal rays, the distal margins of opaque zones were also considered to be annuli, and we considered an annulus to be completely formed when translucent material was visible beyond the distal margin. In pectoral fin ray sections, the pattern in the annual increment deposition was the opposite; annual increments included a relatively wide opaque zone and a narrow translucent zone. Annuli in pectoral fin ray sections were defined as the distal edge of the narrow translucent zones, which were considered completely formed when opaque material was visible beyond the margin.

Age (i.e., the number of completed annuli present) was estimated independently for each structure by two experienced readers. Readers had more than 8 years of professional experience estimating fish age for a variety of species, including Alligator Gars, and from various calcified structures (e.g., otoliths, fin rays, and opercula). Age assignments were made without reference to the ages estimated from other structures, other readers, fish length, year of collection, or knowledge of the expected location of the OTC time stamp. For otoliths, readers examined both structures (when available) prior to assigning an otolith age. Similarly, readers examined all sections of the pectoral fin rays to assign a pectoral fin ray age. Generally, readers assigned an age for the structure based on the otolith or section that contained the most putative annuli because additional annuli were typically found near the outer margin of the structure, suggesting that minor preparation differences were hindering visibility. In all structures, readers considered closely spaced annuli as discrete as long as they did not merge together. Consensus age estimates were assigned to each structure based on either agreement between the two independent estimates or through a reconciled estimate where the two readers viewed the structure together. For each structure, OTC time stamps were not illuminated until after an age was assigned. When present, the location of the time stamp was recorded relative to the nearest annulus (e.g. "on the sixth annulus") and the number of putative post–time stamp annuli were enumerated based on its location.

Data analyses.— Mark efficacy was calculated as the percent of fish with an identifiable OTC time stamp for each calcified structure. For structures with OTC marks, the accuracy of the consensus age estimates was evaluated for each calcified structure and species by calculating the percentage of fish in which the number of post–time-stamp annuli corresponded to the number of annuli expected. The number of expected post–time stamp annuli was based on the amount of time that had elapsed since the fish was injected with OTC. Errors were determined by comparing the observed location and estimated number of post–time stamp annuli with the expected values. For example, if a fish was injected in May 2010 and collected in August 2011, it was expected that the time stamp would be associated with the second-to-last annulus (i.e., 2010) and that there would be growth beyond the last annulus (i.e., 2011). In this example, if the estimated location of the time stamp was associated with the third-to-last annulus (i.e., 2009), the age was considered to be overestimated by 1 year, whereas if it was associated with the last annulus (i.e., 2011), age was considered underestimated by 1 year. Deviations from expected time stamp locations indicated that annuli were not correctly identified, that annuli had not formed, or that discontinuities (i.e., subannular checks) were counted as annuli.

In addition to assessing age estimation errors using the OTC time stamps, we assessed the relative bias associated with each calcified structure by plotting the age estimates derived from pectoral fin ray sections and whole branchiostegal rays against those derived from ground otoliths using age bias graphs (Campana et al. 1995). This analysis was limited to fish for which the number of post–time stamp annuli in otoliths was correctly identified. For these fish, the mean and 95% confidence intervals of the age estimates from pectoral fin ray sections and branchiostegal rays were calculated for each otolith age-class. These data were then plotted relative to a 1:1 line of equivalence to identify any structure-specific bias (Campana et al. 1995).

The precision of the age estimates was evaluated for each calcified structure and species based on the initial age estimates assigned prior to the consensus reads. The metrics used to evaluate precision included the percent agreement between readers and the average coefficient of variation (CV; CV = 100 × SD/mean; Chang 1982; Kimura and Lyons 1991; Campana et al. 1995). Good precision (i.e., CV $\leq$ 5%; Campana 2001) indicates that putative annuli can be consistently interpreted by readers, whereas poor precision suggests that the interpretation of zonation is difficult. We also assessed reader bias for each species and structure using age bias graphs (Campana et al. 1995); for each age-class determined by reader 1, we calculated the mean and 95% confidence intervals of the age estimates from reader 2. These data were then plotted relative to a 1:1 line of equivalence to identify any individual reader bias (Campana et al. 1995).

RESULTS

Accuracy of Age Estimates

The mark efficacy of OTC time stamps and the ability to correctly identify all post–time stamp annuli varied greatly among calcified structures and species. Marking success was highest for ground sagittal otoliths and intermediate for pectoral fin ray sections; OTC marks were not identified in whole branchiostegal rays (Table 1). Mark efficacy was not related to the amount of OTC that was injected. Spotted Gars injected at 100 mg/kg body weight had OTC time stamps in 91.7% of their sagittal otoliths and 62.5% of their pectoral fin rays. Similarly, 91.7% of the otoliths and 61.0% of the pectoral fin rays from Spotted Gars injected at 75 mg/kg body weight were marked. In sagittal otoliths, high magnification was required to identify all annuli and to determine the location of OTC marks in Spotted Gars older than age 10 and Longnose Gars older than age 12 (Figure 2). Later annuli in these fish often occurred very close together along the ventral margin and with low magnification readers tended to underestimate age (Figure 2). As expected, OTC time stamps were generally associated with otolith annuli, suggesting that annulus formation was complete by June, as in other gar species. However, in pectoral fin rays OTC marks frequently occurred along the outer margin in fish up to age 10. In fish older than age 10, OTC marks were absent in 93.8% of the Longnose Gar and 87.5% of the Spotted Gar pectoral fin ray sections. For sagittal otoliths with time stamps, readers correctly identified the location of OTC marks and the number of post–time stamp annuli for 23 Longnose Gars (71.9%; ages 3–25) and 73 Spotted Gars (83.0%; ages 1–27). When incorrect, readers were off by a single year, generally underestimating the time since marking (Table 1). The accuracy of age estimates derived from otoliths declined with age for both species. For Longnose Gars, accuracy was 90.0% through age 5 ($n = 10$), 80.0% for fish ages 6 through 10 ($n = 5$), and 58.8% for fish older than age 10 ($n = 17$). Similarly, for Spotted Gars, accuracy was 95.1% through age 5 ($n = 41$), 81.8% for fish ages 6 through 10 ($n = 22$), and 60.9% for fish older than age 10 ($n = 23$). Based on these data, annual increment periodicity was confirmed in ground sagittal otoliths viewed at high magnification through age 10.

We were unable to confirm annual increment periodicity in pectoral fin ray sections or whole branchiostegal rays because OTC marks largely occurred along the outer margins of pectoral fin rays or were absent altogether. For pectoral fin rays with OTC marks, consensus age estimates frequently underestimated the time that had elapsed since marking (Table 1). In most instances, readers underestimated the time since marking by 2 years ($n = 15$ [71.4% of Longnose Gars]; $n = 31$ [50.0% of Spotted Gars]). Overall, readers only attained an accuracy of 14.3% for Longnose Gars and 22.6% for Spotted Gars (Table 1) and never correctly identified the amount of time since marking for a fish older than age 5. The accuracy of pectoral fin ray age estimates for fish up to age 5 with OTC marks was 33.3% for Longnose Gars ($n = 9$) and 40.0% for Spotted Gars ($n = 35$). Because OTC marks could not be identified in branchiostegal rays, we could not formally assess accuracy for this structure.

Relative to consensus age estimates from sagittal otoliths for which post–time stamp annuli were correctly identified ($n = 23$ for Longnose Gars, 73 for Spotted Gars), age estimates from pectoral fin rays and branchiostegal rays were consistently below the actual ages for both species (Figure 3). Age estimates derived from pectoral fin rays only agreed with those from otoliths for 13.0% of Longnose Gars and 17.8% of Spotted Gars; branchiostegal ray age estimates only agreed for 4.3% of Longnose Gars and 13.7% of Spotted Gars. Fish age was underestimated more severely for branchiostegal rays than for pectoral fin rays. For the oldest age-classes (based on otoliths), branchiostegal rays often underestimated age by more than 15 years (Figure 3).

Precision of Age Estimates

For both Longnose and Spotted Gars, agreement between readers was relatively poor for all structures. Prior to the consensus read, the two readers' age estimates agreed for 39.6% of the otoliths and 43.8% of the pectoral

TABLE 1. Number of Longnose and Spotted Gars for which age was estimated from ground sagittal otoliths (with high magnification), sectioned pectoral fin rays, and whole branchiostegal rays. For each structure, we report the oxytetracycline mark efficacy (*n* with OTC) and the number of structures with an OTC time stamp for which the expected number of post–time stamp annuli was correct, underestimated, and overestimated based on a consensus age estimate.

Structure	*n* fish	*n* with OTC	Correct	Underestimate	Overestimate
			Longnose Gar		
Otolith (115×)	48	32 (66.7%)	23 (71.9%)	7 (21.9%)	2 (6.3%)
Pectoral fin ray	48	21 (43.8%)	3 (14.3%)	17 (81.0%)	1 (4.8%)
Branchiostegal ray	48	0			
			Spotted Gar		
Otolith (115×)	96	88 (91.7%)	73 (83.0%)	13 (14.8%)	2 (2.3%)
Pectoral fin ray	101	62 (61.4%)	14 (22.6%)	46 (74.2%)	2 (3.2%)
Branchiostegal ray	101	0			

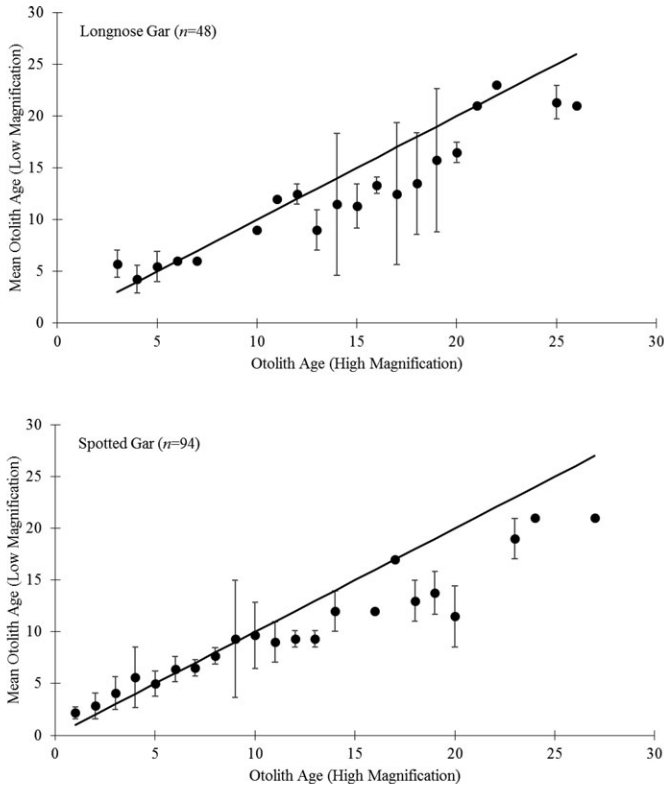

FIGURE 2. Age bias graphs for Longnose and Spotted Gars comparing mean consensus age estimates and 95% confidence intervals from ground sagittal otoliths viewed with low magnification (up to 45×) with estimates from the same structures viewed with high magnification (up to 115×). The 1:1 line provides a reference which indicates that when otoliths were viewed with low magnification readers tended to underestimate the ages of fish older than age 10.

fin rays and branchiostegal rays from Longnose Gars. Estimated ages were within 1 year for 77.1% of otoliths, 66.7% of pectoral fin rays, and 75.0% of branchiostegal rays. Average CVs between the two readers for Longnose Gars were 8.9% for otoliths, 7.1% for pectoral fin rays, and 9.5% for branchiostegal rays. The percentage agreement between readers was slightly better for Spotted Gars, at 54.2% for otoliths, 52.5% for pectoral fin rays, and 66.3% for branchiostegal rays. Age estimates were within 1 year for 79.2% of otoliths and 87.1% for both pectoral fin rays and branchiostegal rays. Spotted Gar CVs were 10.6% for otoliths, 8.4% for pectoral fin rays, and 13.3% for branchiostegal rays. Although precision was generally poor, no consistent reader biases were identified for any of the calcified structures (Figure 4).

DISCUSSION

We attempted to validate annual increment periodicity in three calcified structures that have been used to estimate the ages of Longnose and Spotted Gars. Our results indicate that past age estimates derived from whole branchiostegal rays and sectioned pectoral fin rays likely underestimated individual ages, particularly for fish older than age 5. We were able to validate annual increment periodicity in ground sagittal otoliths from Longnose and Spotted Gars through age 10 (i.e., accuracy ≥80%). However, we found age estimation from otoliths in these species to be particularly challenging and recommend that readers verify their ability to produce accurate age estimates using reference collections (Campana 2001). At this time, we were unable to confirm annual increment periodicity in ground sagittal otoliths from fish older than age 10 because the number of post–time stamp annuli was incorrectly estimated by 1 year in about 40% of the fish. If investigators choose to use age estimates derived from otoliths to make inferences about older age-classes, they should be aware that they may be slightly underestimating the true ages.

The poor mark efficacy in the branchiostegal rays and pectoral fin rays from older fish could have resulted from mark degradation. Bones act as reservoirs for calcium and other minerals that can be readily resorbed or remodeled (Castanet et al. 1993). While resorption or remodeling of the calcium containing the OTC marks could have obscured or degraded the time stamps, our preparation methods may also have contributed to the low proportion of structures with marks. Both pectoral fin rays and branchiostegal rays were cleaned using hot or boiling water. It is unknown whether heat can degrade the fluorescence of OTC marks, as exposure to light does; however, cooking can reduce OTC residues in muscle and to a lesser extent in shell and bone (Maruyama and Uno 1997; Uno et al. 2006).

It is also probable that the poor marking success experienced in this study resulted because calcification did not occur in these bones at the time of marking (i.e., the OTC was not incorporated into the bone). Oxytetracycline binds with calcium and is rapidly incorporated into growth increments where calcification is occurring at the time of marking (Casselman 1987; Campana 1999). Unlike otoliths, which form through accretion and continue to grow throughout the life of the fish (Degens et al. 1969), bones form through osteogenesis. When growing, bones form opaque zones during times of rapid somatic growth and translucent zones during slow growth (Casselman 1974; Castanet et al. 1993). During times of zero somatic growth (in length or mass), annuli may not form in these bones. Many of the fish in this study, particularly the older specimens, did not increase in length or weight, and this lack of growth may have contributed to the inconsistent deposition of OTC marks and annuli in branchiostegal rays and pectoral fin rays. Lack of growth may also have contributed to a similar phenomenon observed in the scales of Rainbow Trout *Oncorhynchus mykiss*,

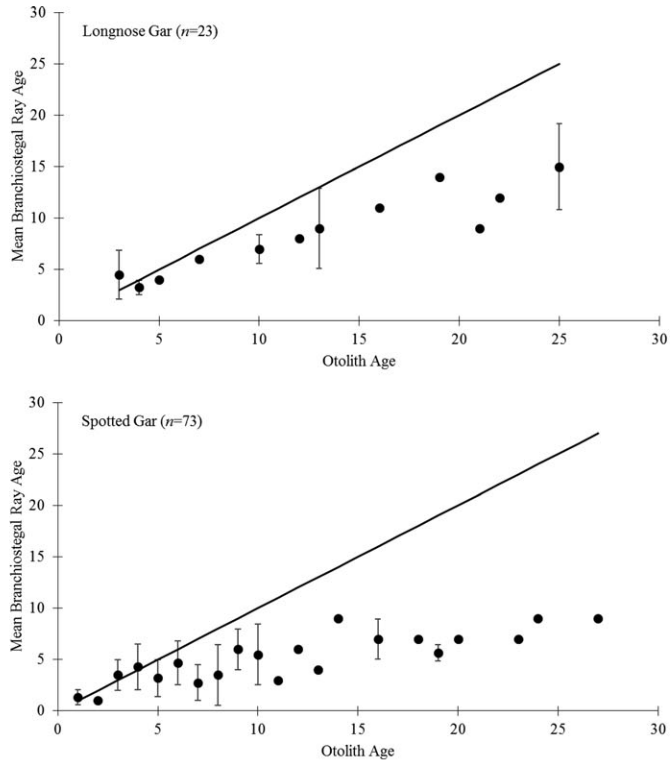

FIGURE 3. Age bias graphs for Longnose and Spotted Gars comparing mean consensus age estimates and 95% confidence intervals from branchiostegal rays and pectoral fin ray sections with estimates from sagittal otoliths for which the number of post–time stamp annuli was correctly identified. The 1:1 reference line indicates that both branchiostegal rays and pectoral fin rays produced underestimates of fish age relative to those from otoliths.

where the proportion of scales with OTC marks declined with age, becoming completely absent by age 3 (Hining et al. 2000).

Regardless of the reason for our poor mark efficacy, it is apparent that ages derived from whole branchiostegal rays and sectioned pectoral fin rays can underestimate the true ages. Both of these calcified structures substantially underestimated age compared with the estimates derived from otoliths, where the number of post–time stamp annuli were correctly identified. Age estimates from these two bones began to diverge from those from otoliths at about age 5, and by age 20 they often underestimated otolith age by more than 10 years. For example, Spotted Gars estimated to be age 20–27 with otoliths were estimated to be a maximum of age 11 with branchiostegal rays. In addition, different interpretations of zonation in these structures could lead to even more severe underestimation of age because we considered closely spaced putative annuli as discrete in all structures. Although universal extrapolation of our findings to past studies is inappropriate because growth may affect the utility of these bones, our results should raise serious concerns about the validity of past age data. Age estimates from these structures likely are systematically biased, suggesting that past estimates of growth and mortality are substantially overestimated, causing much uncertainty regarding published inferences about these species' life history, ecology, and population dynamics. We emphasize these concerns because similar age estimation errors have resulted in the collapse of many valuable marine fish stocks (e.g., Beamish and McFarlane 1983; Lai and Gunderson 1987; Cailliet and Andrews 2008).

Reliable estimates of vital rates and inferences about life history and ecology require that calcified structures provide both accurate and precise age estimates. While we found that sagittal otoliths are capable of producing accurate age estimates (through age 10) for Longnose and Spotted Gars, the precision was lower than desired. The average CVs for ground sagittal otoliths were well above the target of 5% proposed by Campana (2001), although they were within the range typically reported in the age estimation literature. Low precision often reflects the difficulties associated with age estimation, and we faced several challenges in estimating age for these two species from sagittal otoliths. These included not being able to (1) consistently prepare otoliths at the correct angle and depth, (2) discern annuli that were in very close proximity to each other near the ventral edge, (3) distinguish

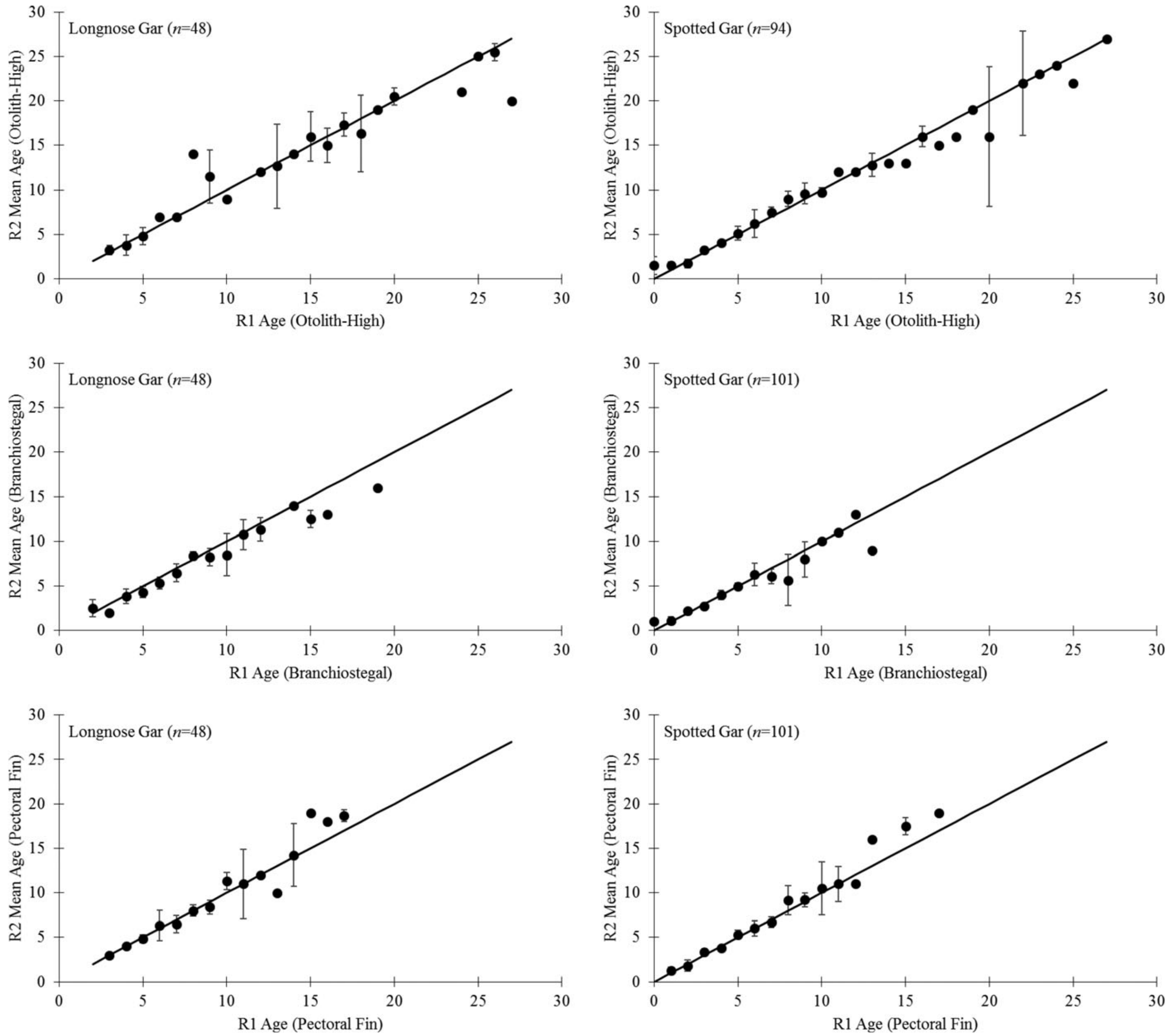

FIGURE 4. Age bias graphs comparing the mean age estimates and 95% confidence intervals from reader 2 (R2) with those from reader 1 (R1) for sagittal otoliths, branchiostegal rays, and pectoral fin ray sections. The 1:1 reference line indicates that there was no reader-specific bias in these age estimates.

between annuli and strong discontinuities (subannular checks) in the first few years of life, and (4) interpret irregular growth patterns (i.e., highly variable increment widths). Like Buckmeier et al. (2012), we found that the quality of preparation was very important to identifying all annuli. When both otoliths were processed, age estimates between otolith pairs for the same individual often differed by 1 or 2 years for older fish. These additional annuli were often visible near the ventral edge of one otolith, suggesting that the area of annulus deposition was limited.

Because of the many difficulties experienced with estimating the ages of Longnose and Spotted Gars from sagittal otoliths, we recommend that only highly experienced personnel be used and that the effects of age estimation error be considered. In this study, we used experienced readers that had previously estimated the ages of Alligator Gars. Less experienced readers would likely have produced age estimates with even lower precision and accuracy. We recommend that prior to attempting to estimate age in wild gars all personnel receive extensive training and become experienced with processing and interpreting annuli using reference otoliths (Campana 2001). Otoliths marked with OTC time stamps can be particularly useful as reference structures with which to train personnel to correctly prepare and identify annuli (Buckmeier et al. 2012). Quality control measures should also be considered to verify that personnel are correctly estimating ages. While validation quantifies a level of accuracy that is attainable, it does not ensure that such a level of accuracy will always be achieved. Based on the findings of this study, investigators should also consider how low precision and a tendency to underestimate the true ages of older fish could affect estimates of age-derived population parameters. Imprecise age data resulting from random errors generally do not significantly alter growth and survival estimates (Ricker 1975), but they can mask recruitment variability by increasing the uniformity across age-classes (Bradford 1991; Kimura and Lyons 1991; Beamish and McFarlane 1995). Systematic errors tend to be more serious, affecting most age-derived population parameters. For example, consistent underestimation of true ages inflates estimates of growth and mortality, biases estimates of longevity and age at maturity downward, and condenses age structure (Lai and Gunderson 1987; Beamish and McFarlane 1995).

In this study we were unable to complete the process of age validation for all age-classes of Longnose and Spotted Gars. Based on our observations, we believe that annual increment periodicity occurs in sagittal otoliths throughout the life of these fish; however, the methods used were insufficient to consistently observe annuli in fish greater than age 10. Our observation that annuli near the ventral edge of otoliths were often missed in one otolith and observed in the other suggests that measurement errors related to otolith processing resulted in the ages of older fish being underestimated. While additional experience may be sufficient to improve the preparation of otoliths from older fish, alternative or more refined preparation methods should be explored in hopes of identifying a method that can increase the consistent visibility of annuli. Such a method would not only improve accuracy, it should also result in greater precision. Some options might include mounting the otoliths in resin to improve consistency in the angle and depth of preparation, cutting serial transverse sections through the otolith to identify the extent of the area of annulus deposition along the ventral surface, and staining or etching the otoliths to highlight annuli. Additional age readers or readings, as suggested by Buckmeier et al. (2012), might also improve accuracy and ultimately increase the precision of age estimates. Until a more reliable method is identified and validated, age estimates derived from otoliths for these species should be used with caution.

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