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Validity of Otoliths and Pectoral Spines for Estimating Ages of Channel Catfish

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Abstract.—Basal recess and articulating process sections of pectoral spines are often used to estimate the age of channel catfish *Ictalurus punctatus*. However, identification of annuli in pectoral spine sections can be difficult. We developed and validated a method for estimating the age of channel catfish by using sagittal otoliths. We also validated a new method using pectoral spines in which a single cut is made through the dorsal and anterior processes (hereafter termed cut spines) and annuli are enhanced with side illumination. Age estimates from otoliths and cut spines were compared with age estimates from traditional articulating process sections of pectoral spines for channel catfish of known ages (1–4). Age estimates by the three methods were correct for more than 90% of fish after two experienced readers independently estimated ages and resolved disagreements by mutual examination. Otoliths were more accurate and less variable in estimating age. Otolith age estimates were always within 1 year and, after mutual examination of the structures in question, 97% of the assigned ages agreed with known age. The accuracy of cut spines and articulating process sections after mutual examination was similar; however, the cut-spine method was simpler than preparing articulating process sections. Otolith annuli were more distinguishable than pectoral spine annuli and were validated for age-1–4 channel catfish. Therefore, we recommend using otoliths to estimate the age of channel catfish.

Historically, basal recess or articulating process sections of the pectoral spine have been used to estimate the age of channel catfish *Ictalurus punctatus* and flathead catfish *Pylodictis olivaris*; however, interpretation of annuli can be difficult (Sneed 1951; Marzolf 1955; Mayhew 1969; Prentice and Whiteside 1975; Turner 1982; Crumpton et al. 1987). As fish age, expansion of the central lumen erodes early annuli, thereby causing the true age of older fish to be underestimated (Muncy 1959; Mayhew 1969). Section location is also important; for example, sections through the basal recess often have fewer annuli than would match the true age of flathead catfish (Turner 1982; Nash and Irwin 1999). Turner (1982) noted that some annuli in spines are composed of multiple growth rings, and Sneed (1951) noted that the irregular arrangement of growth rings presented some difficulties. False marks are usually distinguishable in younger fish because of their proximity to true annuli (Turner 1982); in older fish, however, false marks may become more problematic and lead to overestimation of fish age. Finally, in slow growing and old individuals, spine annuli near the edge tend to merge and may be indistinguishable (Lai et al. 1996; Kocovsky and Carline 2000), thereby increasing the chance of biased age estimates.

Otoliths are valid structures for estimating ages of many freshwater fish species and often are preferred over spines and scales (Taubert and Tranquilli 1982; Erickson 1983; Heidinger and Clodfelter 1987; Hall 1991; Hales and Belk 1992; Hining et al. 2000). However, the use of otoliths for estimating ages of adult catfishes has been limited. Crumpton et al. (1987) reported that otolith annuli were difficult to distinguish in channel catfish, brown bullhead *Ameiurus nebulosus*, and white

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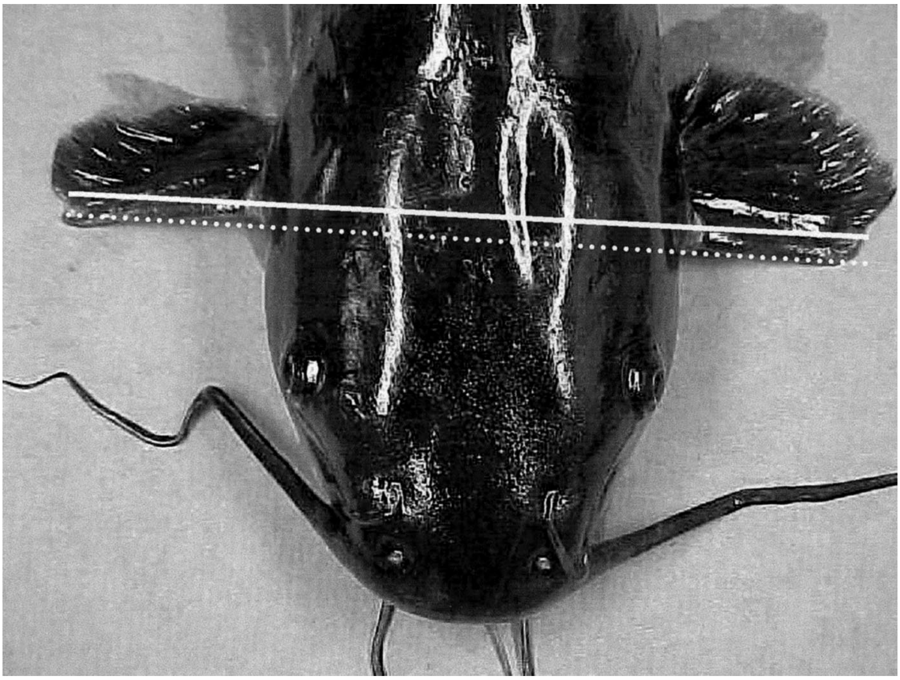

FIGURE 1.—Location of cut used to remove otoliths from channel catfish. The cut (dotted line) is made about 3–5 mm anterior to a line that would connect the locked pectoral spines (solid line).

catfish *A. catus*. However, Nash and Irwin (1999) found otoliths to provide an efficient and accurate method for estimating ages of adult flathead catfish.

Population age structure and growth are measures commonly used to manage fisheries. However, many studies do not consider the accuracy of their age estimates (Beamish and McFarlane 1983). Erroneous age estimates may affect management decisions. Therefore, the relative accuracy of each method should be considered when choosing a method to estimate age. Our objective was to develop and validate improved methods for estimating the age of channel catfish by using otoliths and pectoral spines.

Methods

Channel catfish (N = 225) from the 1996 year-class were acquired from the Alabama Agricultural Experiment Station, Auburn University, in January 1998. Sixteen fish were sacrificed to provide a reference collection of bony structures for estimating age. Adipose fins of the remaining fish were clipped and the fish were stocked into a 0.57-ha pond located at Auburn University. Forage was available in the form of a previously established sunfish *Lepomis* spp. population. Channel catfish were collected and removed from the pond in March, April, May, June, and October 1998 to help determine time of annulus formation. Additional collections were made in July 1999 and 2000. Fish collected represented known-age (1–4) channel catfish.

Channel catfish were collected from the pond by electrofishing and angling. Collection date and total length (mm) were recorded for each fish. Sagittal otoliths were removed by sectioning through the supraoccipital bone using a hacksaw. The cut was made about 3–5 mm anterior to a line that would connect the locked pectoral spines (Figure 1). Otoliths were then removed from the posterior portion of the skull by forceps. If otoliths were difficult to locate, the top of the skull was removed with wire cutters to provide better access. Otoliths were stored and dried in vials marked with reference numbers. Pectoral spines were disarticulated and removed (Mayhew 1969). Spines were cleaned and stored in coin envelopes marked with reference numbers.

Because otoliths had not yet been successfully used to estimate the age channel catfish, we experimented with several processing methods—including those of Maceina (1988), which were used by Nash and Irwin (1999) to age flathead catfish, and those suggested by Bagenal and Tesch (1978), which were used by Heidinger and Clodfelter (1987) to age smallmouth bass *Micropterus dolomieu*, walleye *Stizostedion vitreum*, and striped bass *Morone saxatilis*. Otoliths were not used to estimate ages of all fish collected because some otoliths were destroyed during method development.

We experimented with alternative processing methods for pectoral spines to reduce the influence of the central lumen and to improve the recognition of annuli. Ideal section location was determined by cutting multiple cross sections (75 μm thick) of several pectoral spines with a Buehler low-speed isomet saw. Sections for each spine were mounted on microscope slides and viewed through a dissecting microscope (5–25×). Each section was examined and the number of annuli and the relative size of the central lumen were noted. Once the ideal location was determined, we experimented with side illumination (similar to the method for otoliths described by Heidinger and Clodfelter 1987) to improve annulus recognition.

We prepared and examined articulating process sections of pectoral spines (similar to the method described by Turner 1982) to compare age estimates from articulating process sections with age estimates from otoliths and pectoral spines. Articulating process sections were viewed by using transmitted light and a dissecting microscope (5–25×) for channel catfish ages 1–3.

For each structure and preparation method, spines or otoliths from fish of unknown age were combined with structures of known age to reduce bias. Two readers, each with numerous years of experience estimating fish age, independently estimated the ages of structures without reference to fish length. Disagreements were resolved by mutual examination of the questionable structures (hereafter termed a concert reading). Spines and otoliths from fish of unknown age were not included in analyses. Percentage reader agreement was calculated for each method to compare precision. The percentage of correctly estimated fish ages was calculated for each method to compare accuracy. Age bias graphs (similar to those of Campana et al. 1995) were generated for each method of estimating age and were used to assess individual reader bias. In addition, age bias graphs were constructed after a concert reading to determine whether the use of a concert reading corrected any individual bias associated with each method.

The implications of the error associated with each method were assessed by comparing actual mean length at age and age structure with the estimates by each reader and those following a concert reading. Actual mean length-at-age was determined by calculating the mean of the individual lengths at capture for each age-class. Age structure was the number of fish of known age collected from each age-class. Mean length-at-age and age structure estimates, by each reader and following a concert reading, incorporated age estimation error. Comparisons of estimates and actual values would reveal whether age estimation error could potentially alter management decisions. Correct age assignments for all fish in a sample would thus yield estimates of mean length-at-age and age structure that were identical to actual values.

Results

Sample Preparation

Annuli were not consistently visible in channel catfish otolith sections prepared by the methods of Maceina (1988), but they were visible in channel catfish otoliths prepared and viewed by methods similar to those of Heidinger and Clodfelter (1987). Some modifications were necessary because annuli were difficult to discern. Burning otoliths by placing them directly on a hotplate (Corning PC 351; Corning Industries, Corning, New York) changed their color from opaque white to yellowish brown (after 30–60 s at medium heat) and enhanced annular marks. Mounting each burned otolith on a microscope slide by using an adhesive (Crystalbond; Buehler, Lake Bluff, Illinois) allowed easier processing, given the very small size of channel catfish otoliths (Figure 2). To ameliorate the brittleness of channel catfish otoliths, support was provided by forming adhesive around the lower half of the otolith. Otoliths were mounted with the rounded anterior edge upright and the pointed posterior edge in contact with the microscope slide. The anterior–posterior axis was perpendicular to the slide; otherwise, visibility of annuli decreased. Once the adhesive hardened (30–60 s), we hand-sanded the otolith with wetted 600-grit carborundum sandpaper until 1/3–1/2 of the otolith was sanded away revealing the nucleus in the transverse plane (Figure 2). Coating the sanded surface with mineral oil and using side illumination enhanced the appearance of annuli as viewed with a dissecting microscope (10–60×). We found that side illumination with a 1-mm-diameter, single-strand fiber optic filament connected to a light source (Fiber-Lite model 180;

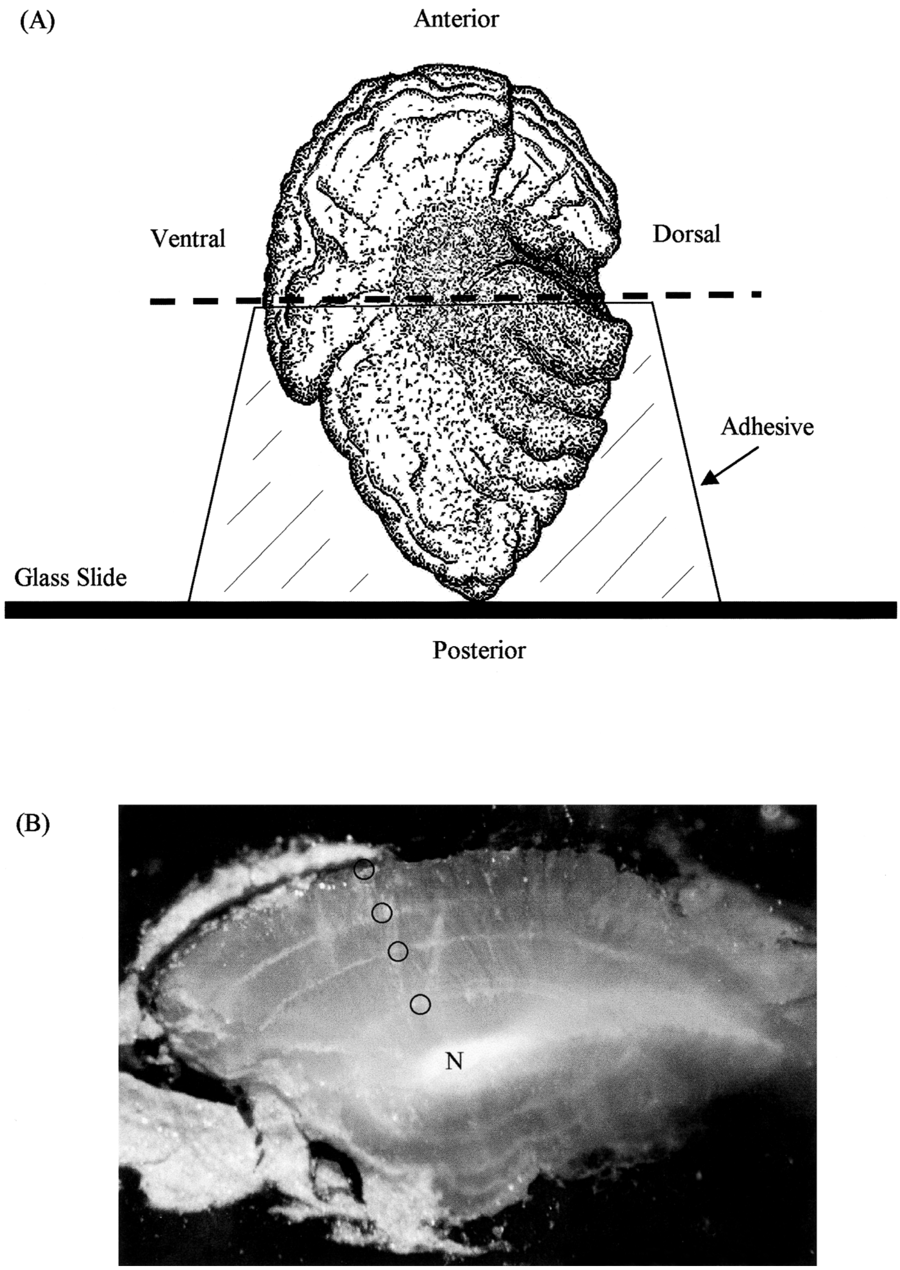

FIGURE 2.—**(A)** Illustration of the right otolith from a channel catfish depicting how it is mounted to a glass slide. The otolith is sanded from the anterior tip to the dashed line, resulting in the transverse view through the nucleus shown in the photograph. **(B)** The photograph of an otolith is from a 4-year-old channel catfish shows the location of the nucleus (N) and annuli (circled); the fourth annulus is located at the edge.

Dolan-Jenner Industries, Inc., St. Lawrence, Massachusetts) provided the best results. Reflecting light at several angles, including through the otolith and at the sanded surface, enabled observation of all annuli.

For pectoral spines, a single cut through the dorsal and anterior processes (Figure 3) provided the best age estimates with the least effort. Serial sections of spines revealed that as early as age 1, annuli were missing in some basal recess sections.

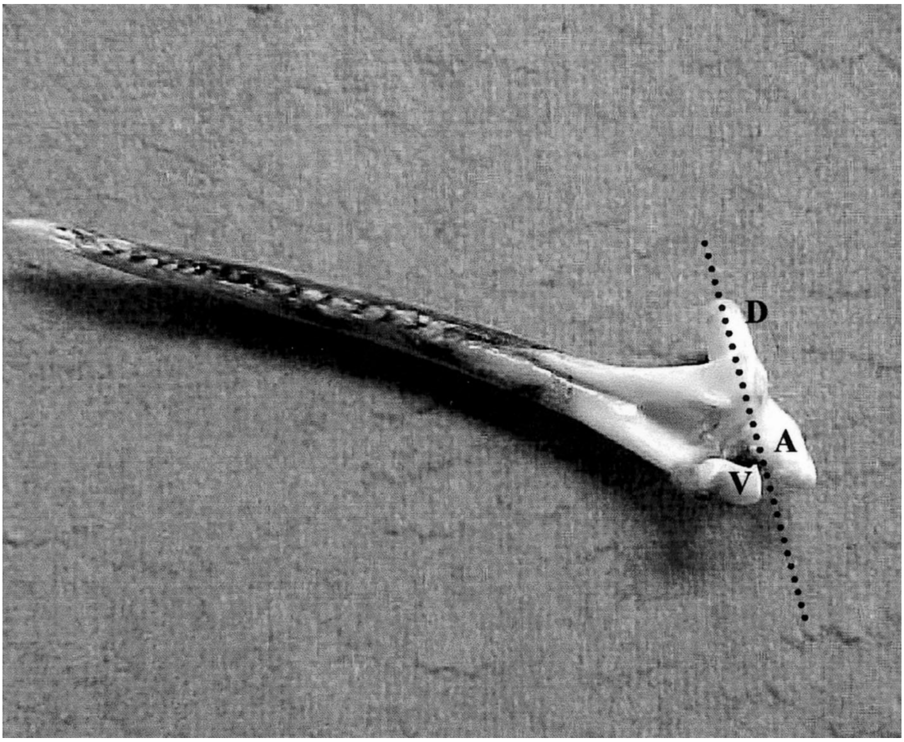

FIGURE 3.—Location of the cut (dotted line) used to process cut pectoral spines for estimating the age of channel catfish. The saw blade should be aligned from the tip of the ventral process (V) to the outer edge of the dorsal process (D) and section through the dorsal and anterior (A) processes. This location minimizes the influence of the central lumen.

The central lumen was smallest in a section taken through the dorsal and anterior processes. We found that this cut could easily be made by mounting a spine in a small hobby vise with rubber jaws and cutting the spine with a hand-held jeweler's saw equipped with a 5/0 blade. Embedding the distal end of the spine in clay and coating the cut surface with mineral oil enhanced visibility of annuli when viewed through a dissecting microscope (5–25×). As with otoliths, a 1-mm fiber optic filament provided effective side illumination. Hereafter this procedure is termed the cut-spine method.

Validation

Annulus formation started in late April and was complete by early June. Otoliths provided the most accurate age estimates of 1–4-year-old channel catfish for each reader and following a concert reading (Table 1). The first and second readers correctly estimated the age of 82% and 95% of the otoliths, respectively. Otoliths in which age was improperly estimated by the readers were always within 1 year of known age (Figure 4). Following concert readings, only one age estimate was incorrect; an age-4 fish was estimated to be age 5. Agreement between readers was 79%. Both readers indicated the first annulus was the most difficult to detect.

Cut-spine and articulating process section age estimates were more variable between readers than were otolith age estimates (Table 1). Agreement between readers was slightly better for cut spines (65%) than for articulating process sections (52%). Reader error ranged from 1 to 6 years (Figure 4). The first reader was consistently less accurate (Table 1), even though both readers were experienced at estimating fish age. After concert readings, however, age estimates from cut spines and articulating process sections were within 2 years of the known age, and more than 90% were correctly estimated. With a concert reading, ages estimated from cut spines and articulating process sections were adequate for 1–3-year-old channel catfish. However, age estimates of age-4 fish exceeded the known age by 1–2 years.

TABLE 1.—Percentages of otoliths, cut pectoral spines, and articulating process (AP) sections for which ages were estimated correctly by each reader and following a concert reading, as well as the precision (percent agreement between readers) for known-age channel catfish (ages 1–4).

		Accuracy			
Age	N	Reader 1	Reader 2	Concert reading	Precision
Otoliths					
1	13	85	92	100	85
2	9	67	89	100	56
3	14	100	100	100	100
4	3	33	100	67	33
Overall	39	82	95	97	79
Cut spines					
1	28	68	100	100	68
2	18	89	94	89	83
3	16	38	94	94	38
4	3	0	0	0	67
Overall	65	63	94	91	65
AP sections					
1	28	68	96	100[a]	64
2	18	72	83	94	56
3	16	25	88	81	25
Overall	62	56	94	94	52

[a] One structure was omitted because readers could not reach agreement.

Age bias graphs revealed that the first reader consistently overestimated the age of channel catfish when using cut spines and articulating process sections; however, his age estimates based on otoliths were unbiased (Figure 4). Often, annular marks on cut spines and articulating process sections consisted of multiple growth rings, resulting in overestimates of age by the first reader. Accessory marks were not observed on otoliths. No bias was noted for the second reader or for age estimates based on a concert reading.

Estimates of mean length at age and age structure based on age assignments by the individual readers often differed from actual mean length at age and age structure (Table 2). For example, using cut spines, the first reader estimated that the population consisted of seven year classes when only four existed, and the abundance of age-3 fish was drastically underestimated. Following a concert reading, otoliths provided estimates of mean length-at-age and age structure similar to actual values for age-1–4 fish; spines yielded similar values for age-1–3 fish.

Discussion

After concert readings, age assignments were accurate for age-1–4 channel catfish as determined by using otoliths and for age-1–3 channel catfish as based on pectoral spines. However, individual interpretation of annuli was more variable for pectoral spines. Prentice and Whiteside (1975) also noted much variability between readers who were estimating ages of channel catfish spine sections. Conversely, reader agreement for otolith age assignments was high, indicating annuli on otoliths were easier to identify. Annuli on otoliths have also been determined to be more distinguishable than spine annuli for flathead catfish (Nash and Irwin 1999) and walleye (Erickson 1983; Kocovsky and Carline 2000). Although channel catfish otolith annuli were distinct, they were generally lighter than in other fishes such as largemouth bass *Micropterus salmoides*, crappie *Pomoxis* spp., and flathead catfish. With practice, annuli on channel catfish otoliths were easily identified.

The error from compounding sources probably makes spines an improper choice for estimating ages of old channel catfish, especially in slow-growing populations. We found that annular marks composed of multiple rings led to overestimation of age. Presumably, identification of accessory growth rings would be more difficult as fish age. Double rings have also been mistakenly identified as separate annuli in dorsal spines of walleye (Kocovsky and Carline 2000). Conversely, several authors (Erickson 1983; Welch et al. 1993; Nash and Irwin 1999) have reported that spine ages can underestimate the age of old fish. Most often, expansion of the central lumen and the inability to separate annuli near the edge of the structure are cited as explanations of missing annuli. Kocovsky and Carline (2000) reported difficulty in estimating ages from sections of dorsal spines from walleye because of merged rings, partial rings, and crowding of distal rings; they attributed such structural abnormalities to slow growth. Accessory marks were not observed in the channel catfish otoliths we examined, and many of the difficulties associated with estimating ages of spines are not relevant to otoliths. Therefore, otoliths may provide accurate age estimates for channel catfish older than age 4.

Inconsistencies resulting from variable interpretation can lead to improper age assignments, even for validated methods of estimating age (Lai et al. 1996). Both readers in our study had numerous years of experience determining fish age; however, their relative accuracy differed. Thus, reader experience may not be a good indicator of reader accuracy. Erroneous age estimates, to the extent observed for the first reader when using pectoral spines, may be sufficient to result in inappropriate

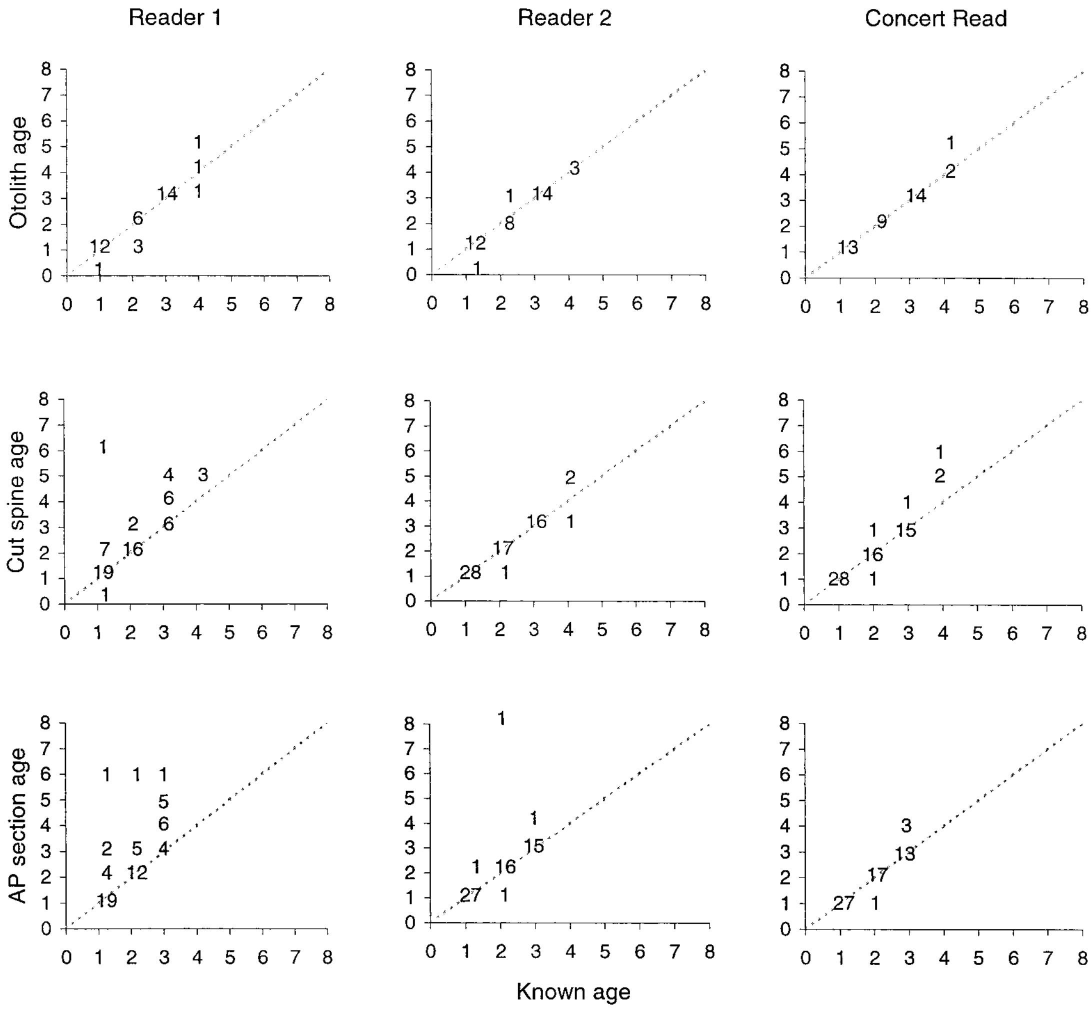

FIGURE 4.—Age bias graphs for reader 1, reader 2, and a joint (concert) reading for age estimates of channel catfish from otoliths, cut pectoral spines, and articulating process (AP) sections. Numbers indicate sample sizes. The dashed line represents agreement between age estimates and known ages.

management decisions. The use of multiple readers and a concert reading in our study reduced reader bias and provided accurate data. Ideally, individuals should verify that they are correctly identifying annuli by using known-age fish whenever possible. However, if known-age fish are not available, our results suggest the use of multiple readers and a concert reading may ensure data accuracy.

We believe that otoliths are the best structure for estimating age of channel catfish. We did not measure processing time for each method evaluated; however, the additional time required to collect and prepare otoliths versus cut spines was relatively small. Both methods were faster than preparing articulating process sections. In addition, we believe that sacrificing a sample of fish to provide age estimates will not significantly affect most channel catfish populations. In instances when fish cannot usefully be sacrificed, cut pectoral spines may provide an alternative method for estimating the age of channel catfish of age 3 or younger. However, a sample of otoliths should initially be used to confirm ages estimated from cut spines. Regardless of the method, some attempt should be made to verify that annuli are being identified correctly.

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TABLE 2.—Comparison of actual and estimated mean total lengths (TL) from otoliths, cut pectoral spines, and articulating process (AP) sections. Each column represents the same group of fish, with the number of age structures in parentheses.

Age	Actual mean TL	Estimate		
		Reader 1	Reader 2	Concert reading
		Otoliths		
0		325 (1)	331 (1)	
1	321 (13)	318 (15)	320 (12)	321 (13)
2	323 (9)	328 (6)	326 (8)	323 (9)
3	368 (14)	374 (15)	364 (15)	368 (14)
4	452 (3)	424 (1)	452 (3)	443 (2)
5		471 (1)		471 (1)
		Cut spines		
0		311 (1)		
1	321 (28)	320 (19)	320 (29)	320 (29)
2	335 (18)	331 (23)	337 (17)	335 (16)
3	370 (16)	363 (8)	375 (17)	371 (16)
4	452 (3)	362 (6)		352 (1)
5		412 (7)	448 (2)	448 (2)
6		325 (1)		462 (1)
		AP sections		
1	321 (28)	322 (19)	320 (28)	322 (28)
2	335 (18)	339 (16)	334 (17)	335 (17)
3	370 (16)	332 (11)	370 (15)	369 (13)
4		380 (6)	374 (1)	372 (3)
5		371 (5)		
6		326 (3)		
7				
8			367 (1)	

proved this manuscript. We thank the staff of the Alabama Cooperative Fish and Wildlife Research Unit for collecting fish and removing aging structures. The Alabama Agricultural Experimental Station, Auburn University, furnished fish and the pond. The otolith illustration in Figure 2 was drawn by R. G. Howells. Funding was provided by the Alabama Department of Conservation and Natural Resources, Game and Fish Division, through Federal Aid in Sport Fish Restoration Grant F-40-30 and the Texas Parks and Wildlife Department.

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