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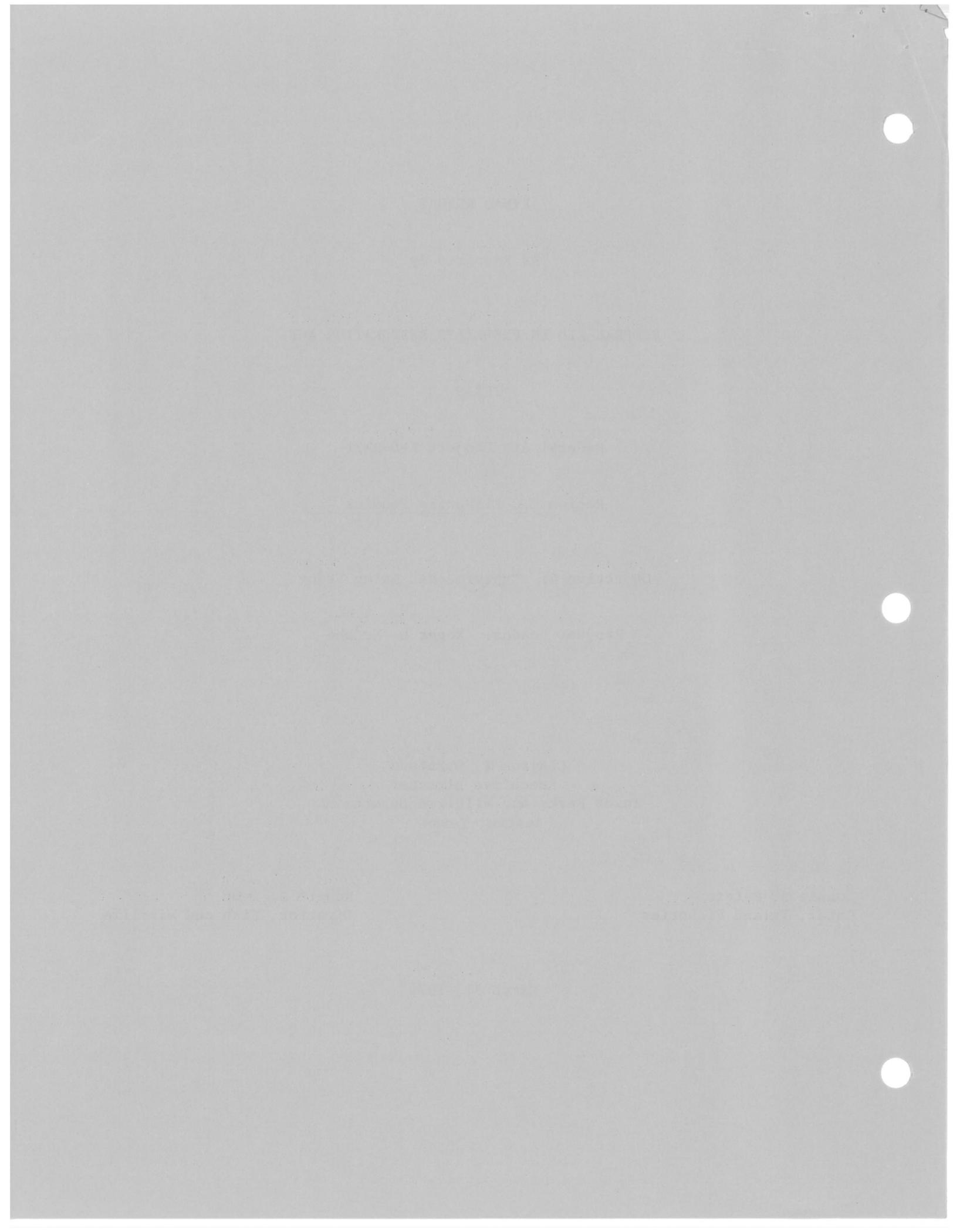
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A Comparative Evaluation of Methods for
Aging Fishes in Lake Corpus Christi, Texas

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ABSTRACT

This study compares the usefulness of scales, vertebrae, otoliths, opercular bones, and pectoral spines for aging studies in southern Texas. The agreeability of age determinations obtained by two observers was determined for 12 species of fishes from Lake Corpus Christi. Statistical analyses indicated scales and otoliths were the preferred structures for aging scaled fishes and vertebrae, opercular bones, and pectoral spines were the best structures for aging catfishes.

INTRODUCTION

The value of age determination of fishes has been recognized by investigators since the late 1800's (Van Oosten, 1923; McConnell, 1951). The ability to age fish provides biologists with information such as: (1) determination of growth rates, (2) time of sexual maturity, (3) age at which a given species reaches catchable size, (4) environmental unsuitabilities affecting growth, (4) fluctuation of normal age composition of fish populations from year to year, (6) the suitability of subsequent stocking (Lagler, 1956).

Techniques generally used for fish aging have been described by Van Oosten (1929), Menon (1950), Rounsefell and Everhart (1953), Lagler (1956), Chugunova (1963), Tesch (1968) and others. The main methods of determining age are analysis of length frequency data and the counting of seasonal rings or zones in various hard body parts of fishes. The first method provides fairly precise estimates of age composition of fish populations when large samples are taken. The second method provides the age of individual fishes, but precision of readings depends on the experience of the reader, region of country fishes are collected from, size of focus, sample size, degree of scale absorption, recognition of false annuli, etc.

Most aging studies have been confined to northern states where seasonal temperature differences are distinct. Growth of fishes in these areas stops during the winter and starts in the spring when the water is warm. Usually a well formed annulus or year mark is formed at this time. In the southern part of the United States, the winter season is characterized by periods of summer-like weather which may cause false annuli formation. This problem has discouraged age and growth studies in the South. But, because of the importance of these studies in the fisheries management field, this study was undertaken to find aging methods that might be used successfully in Texas.

Fish samples were collected from Lake Corpus Christi--a 22,000-acre reservoir located at 28° 5' N and 97° 28' W or about 4 miles southwest of Mathis, Texas on the Nueces River. The lake was built in 1929 (volume = 300,000 acre-feet; drainage area = 16,656 square miles). It is a shallow lake that does not thermally stratify.

METHODS AND MATERIALS

Aging structures from 1,316 fishes (12 species) were collected from 1968 to 1971. Three species of catfishes [blue catfish (*Ictalurus furcatus*), channel catfish (*Ictalurus punctatus*), and flathead catfish (*Pylodictus olivaris*)] and nine species of ctenoid scaled fishes [white bass (*Morone chrysops*), largemouth bass (*Micropterus salmoides*), warmouth (*Lepomis gulosus*), reardear sunfish (*Lepomis microlophus*), bluegill (*Lepomis macrochirus*), longear sunfish (*Lepomis megalotis*), white crappie (*Pomoxis annularis*), black crappie (*Pomoxis nigromaculatus*), and freshwater drum (*Aplodinotus grunniens*)] were used in the study.

Fishes were captured with experimental gill nets, beach and bag seines, a bottom trawl, and wire traps. Catches were worked fresh or placed in ice and worked within 24 hours after capture. The species name, date of capture, standard and total lengths (mm) and weight (gms) were recorded on individual specimen envelopes.

Age estimates were derived from seasonal rings or zones on scales and/or other hard parts. Scales, vertebrae, otoliths and opercular bones were used in estimating the ages of scaled fishes while pectoral spines, vertebrae and opercular bones were used in estimating the ages of catfishes. Collecting, aging and analysis procedures used for each type of structure are given below.

Scales: Approximately 20 scales were taken from the left side of the body below the anterior portion of the dorsal fin. Scales were removed by scraping them free with a scalpel. Next, they were placed between two layers of absorbent paper and stored in a specimen envelope.

Before reading, scales were cleaned by washing in warm, soapy water. Impressions of the scales were then made on 1" x 3" x 0.040" cellulose acetate slides with the aid of a heated hydraulic press similar to the apparatus described by Greenbank and O'Donnell (1948).

Scale impressions were projected on a screen with a Model X-1000 Ken-A-Vision microprojector equipped with a 32mm Macro-Tessor lens of 100X magnification. Scale measurements (mm) were taken along an antero-lateral axis from the focus to the outermost point of one corner of the anterior scale margin.

True annuli were determined by the following criteria: (1) crossing over of incomplete circuli distally by a continuous circulus in the lateral field (Rounsefell and Everhart, 1953; Lagler, 1956; Carlander, 1961; Chugunova, 1963; Tesch, 1968), (2) a mark preceded by closely spaced circuli proximally followed by widely spaced circuli distally (Rounsefell and Everhart, 1953; Lagler, 1956; Carlander, 1961), (3) a wide space between series of circuli (Carlander, 1961; Chugunova, 1963), (4) an evident mark continuing as a dark band across the

posterior field separating older, blunt ctenii from newer, sharp ctenii (Miller, 1966) and (5) marks where radii bend or end at their juncture (Tesch, 1968). The first three criteria were relied upon most heavily, while the last two were considered less important for designating true annuli.

Criteria for determining these annuli were: (1) less distinct and/or less continuous marks than true annuli (Rounsefell and Everhart, 1953; Chugunova, 1963), (2) marks in close proximity to true annuli (Rounsefell and Everhart, 1953), (3) zones of relatively widely spaced circuli proximal to the focus and closely spaced circuli distally (Sprugel, 1954; Chugunova, 1963), (4) lack of extensive crossing over of circuli and limited extension of the marks across the posterolateral field (Sprugel, 1954; Laakso and Cope, 1956), (5) marks illogically occurring in an area representing rapid summer growth (Laakso and Cope, 1956; Chugunova, 1963), (6) discontinuous circuli distal to year marks (Regier, 1962), and (7) failure of marks to appear on all scales observed (Sprugel, 1954; Miller, 1966). Scale marks identified as false annuli did not meet all of these criteria, but usually more than one was associated with these marks.

Vertebrae: Five anterior trunk vertebrae were removed from each fish with surgical scissors or metal snips. The vertebrae were placed in small wire baskets along with other bone structures (operculars and spines) removed from the same fish. The structures were heated at a temperature slightly below boiling in a water vat for approximately 5 minutes to remove unwanted tissue and bones.

Whole vertebrae were placed in petri dishes containing anise oil which made the marks more pronounced. Structures examined dry, in glycerol, xylol, isopropol alcohol, or water showed less distinct marks than those in anise oil. These parts were examined with the aid of a variable-power binocular microscope of 10X to 15X magnification. Reflected and transmitted light were used for illumination.

Criteria for determining true annuli in vertebrae were narrow dark bands concentric with the rim of the centrum that separated broader light colored zones (Lewis, 1948; Applegate and Smith, 1950), and dark bands accompanied by a ridge on the surface of the centrum. False annuli were identified as faint or incomplete marks without a ridge on the centrum's surface.

Otoliths: Of the three pairs of otoliths (ear stones) present in the inner ear of all teleost fishes, the two largest were used for making age estimates in this study. The otolith method was not utilized for catfishes because of the small otolith size and the difficulty encountered in their removal.

Otoliths were collected by removing the lower jaw of each fish with surgical scissors or metal snips. This left the ventral side of the neurocranium floor unobstructed. By scraping the floor tissue away with a scalpel, the enlarged saccular area containing otoliths was located. The sacculus was opened to expose the otoliths for removal. Otoliths were allowed to air dry before being stored in envelopes.

Otoliths were observed whole, with the exception of those from freshwater drum. Due to the thickness of drum otoliths, fracturing was necessary to reveal all year marks. A small chisel and hammer were used to break the otoliths through

the nucleus, perpendicular to the long axis. A variable-power binocular microscope (8X to 12X magnification) was used for examining otoliths. Whole otoliths were viewed on their distal (concave) surface and broken otoliths on their cross-sectioned surface. To aid in identification of marks, anise oil was used as a clearing agent for otoliths, and the structures were placed on a black background and viewed with the aid of reflected light (Schott, 1968). A narrow opaque band, accompanied by a broad translucent band proximally was considered one year's growth. Age was determined by counting the number of white bands present.

Opercular bones: The left opercular bone was used for age determination in all 12 species of fishes studied. The opercular assembly was removed from each fish by cutting anteriorly from the dorsal attachment to the eye orbit, ventrally to the corner of the mouth, and posteriorly along the branchiostegal rays. Removing the entire assembly insured getting the opercular bone undamaged. The opercular assemblies were cleaned in a hot water bath to allow the easy removal of unwanted bone and skin (Le Cren, 1947; and McConnell, 1951).

Growth zones appeared as opaque and translucent bands running parallel to the margin of the dorsal, posterior, and ventral portions of the opercular bone. Broad opaque zones were periods of rapid summer growth and narrow translucent bands represented slow winter growth. Marks showing a gradual change from opacity to transparency and ending abruptly at the beginning of another opaque zone were treated as false annuli. Faint or incomplete marks occurring only along the posterior margin were also judged as false annuli.

The proximal side of the whole opercular structure was viewed for making age estimates. Annuli were most distinct when viewed with reflected light against a dark background. A variable-power, binocular microscope (7X to 10X magnification) was used for viewing operculars. Also anise oil was used as a clearing agent.

Pectoral spines: Pectoral spines were selected for aging purposes since they have been used successfully in studies of channel catfish (Sneed, 1950; Hall and Jenkins, 1952; Marzolf, 1955; Muncy, 1959; Ambrose and Brown, 1971). Spines have also been successfully used for aging blue catfish (Kelly, 1968), and sturgeon (Probst and Cooper, 1955; Chugunova, 1963).

Only the left pectoral spine was used for aging in this study. The spine was removed by cutting through the pectoral girdle, on each side of the articulation, with scissors or metal snips. Unwanted bones and flesh were removed by the hot water method as already described. Spines were air dried and stored in envelopes.

Pectoral spines were mounted in a fast-drying, clear plastic before cross-sectioning to reveal growth marks. This plastic cast provided a larger, more easily handled object during the sectioning process. It also prevented spines from splintering.

A section of spine, approximately 0.2-inch thick, was obtained distal to the basal groove. Both surfaces of the section were polished with abrasive belts mounted on a lapidary wheel. This technique resulted in bone sections that were thin

enough to allow light transmittance, yet durable enough for handling and storage.

Spine sections were observed under reflected light through a variable-power binocular microscope. Anise oil was used as a clearing agent.

Age was determined by counting translucent winter rings. False annuli were identified by their faint or incomplete appearance.

Statistical Analyses: Four scale readers were used at the beginning of the study, but project transfers allowed only two of the original observers to complete the investigation. Statistical analyses are based on the observations made by these two individuals. They were fisheries biologists who read and reread the various aging structures until two readings came out the same. These readings were made independently of each other.

Aging data from the two observers were compared by correlation and F-test statistics to determine their degree of association; that is, the extent their observations agree with one another. Of course, correlations were made according to species and method used in aging. Analysis of variance tests were used to define significant, statistical differences between aging techniques.

RESULTS

Scaled Fishes

Mean ages obtained by two observers aging the same set of fish were similar for a given aging method (scales, vertebrae, otoliths or opercular bones), but mean age comparisons between methods showed less agreement (Table 1). Results of the analysis of variance testing for differences in the mean ages between aging methods and observers revealed a statistical difference between methods, but not between observers (Table 2). The resulting sums of squares analysis for the analysis variance indicated two non-significant groups of aging methods (Table 3). Vertebrae, otoliths, and opercular bones formed one group; and they had a smaller coded age mean than scales. Note no statistical difference was found between opercular bones and scales (the second group). In other words, this analysis does not clearly establish the differences in these methods of aging fishes.

Correlation coefficients obtained by comparing the estimated ages of scaled fishes made by the two readers are given in Table 4. The corresponding z-transformations of the correlation coefficient are also provided. The analysis of variance of z-transformations (Table 5) and the resulting simultaneous sums of squares test of the means of correlation coefficients indicate a significant statistical difference between two groups of aging methods (Table 6). Overlap between the two groups does not permit a clear assignment of the group which vertebrae should be assigned to. However, the correlation coefficients for otoliths ($r = 0.916$) and scales ($r = 0.919$) as compared to operculars ($r = 0.763$) and vertebrae (0.821) indicate the first two are the most consistent aging techniques. Aging by either of these two methods should give a correlation coefficient between 0.856 and 0.955 95 per cent of the time.

TABLE 1. Mean age (\pm standard error of the mean) obtained by two observers aging the same set of fish by four different methods, Lake Corpus Christi data, 1968-1971.

Species	Method ¹	n	Mean first observer	Mean second observer
Bluegill	1	81	1.26 \pm 0.21	1.27 \pm 0.20
	2	81	1.09 \pm 0.18	1.41 \pm 0.16
	3	80	1.36 \pm 0.13	1.32 \pm 0.14
	4	78	0.86 \pm 0.17	1.01 \pm 0.18
Longear sunfish	1	30	1.87 \pm 0.28	2.00 \pm 0.30
	2	33	1.06 \pm 0.23	0.97 \pm 0.25
	3	33	1.24 \pm 0.19	1.36 \pm 0.23
	4	32	1.34 \pm 0.26	1.06 \pm 0.24
Redear sunfish	1	192	1.69 \pm 0.13	1.68 \pm 0.13
	2	194	1.51 \pm 0.10	1.62 \pm 0.11
	3	195	1.67 \pm 0.11	1.67 \pm 0.10
	4	185	1.49 \pm 0.12	1.50 \pm 0.11
White crappie	1	194	1.69 \pm 0.11	1.64 \pm 0.11
	2	199	1.43 \pm 0.11	1.47 \pm 0.12
	3	193	1.23 \pm 0.11	1.31 \pm 0.12
	4	189	1.59 \pm 0.12	1.69 \pm 0.13
Black crappie	1	114	2.28 \pm 0.17	2.32 \pm 0.18
	2	115	1.84 \pm 0.17	1.78 \pm 0.20
	3	115	1.77 \pm 0.18	1.70 \pm 0.20
	4	108	1.98 \pm 0.17	1.91 \pm 0.20

TABLE 1. (Cont.)

Species	Method ¹	n	Mean first observer	Mean second observer
Largemouth bass	1	135	1.57 ± 0.20	1.53 ± 0.20
	2	139	0.97 ± 0.14	1.00 ± 0.14
	3	134	1.00 ± 0.14	1.01 ± 0.14
	4	124	1.06 ± 0.16	1.18 ± 0.16
White bass	1	162	1.90 ± 0.14	1.89 ± 0.14
	2	159	1.00 ± 0.10	1.03 ± 0.11
	3	156	0.97 ± 0.10	1.04 ± 0.11
	4	150	1.12 ± 0.12	1.31 ± 0.13
Warmouth	1	20	2.15 ± 0.44	2.15 ± 0.36
	2	21	1.38 ± 0.26	1.38 ± 0.26
	3	20	1.70 ± 0.26	1.75 ± 0.26
	4	18	1.67 ± 0.28	1.72 ± 0.30
Freshwater drum	1	130	1.78 ± 0.13	1.77 ± 0.14
	2	128	2.57 ± 0.20	2.78 ± 0.22
	3	134	2.63 ± 0.30	2.71 ± 0.31
	4	122	2.43 ± 0.22	2.81 ± 0.26

¹ Method: 1 = scales; 2 = vertebrae; 3 = otoliths; 4 = opercular bones.

TABLE 2. Results of analysis of variance of coded mean ages obtained by each observer using four different methods of aging scaled fishes (data transformed by taking the square root of the coded mean ages).

Source of variation	Sum of squares	df	MS	F
Method	4.809	3	1.6030	5.497*
Observer	0.815	1	0.8150	2.794
Interaction	0.531	3	0.1768	0.606
Error	18.665	64	0.2916	
Total	24.820	71		

* 0.05

TABLE 3. Results of a simultaneous sums of squares analysis for statistical differences (0.05 level) in aging methods used for scaled fishes (Underlined methods are non-significant from each other).

Method	Vertebrae	Otoliths	Operculars	Scales
Coded \bar{X}	1.497	1.611	1.696	1.964

TABLE 4. Correlation coefficients obtained for two observers aging the same set of scaled fish, () equals Z transformation of the correlation coefficient.

Species	Method			
	Scales	Vertebrae	Otoliths	Operculars
Bluegill	0.953 (1.863)	0.710 (0.887)	0.916 (1.564)	0.818 (1.151)
Longear sunfish	0.428 (0.458)	0.786 (1.061)	0.691 (0.850)	0.602 (0.696)
Redear sunfish	0.950 (1.832)	0.819 (1.154)	0.918 (1.576)	0.687 (0.842)
White crappie	0.867 (1.321)	0.737 (0.944)	0.903 (1.488)	0.749 (0.971)
Black crappie	0.912 (1.539)	0.812 (1.133)	0.927 (1.637)	0.810 (1.127)
Largemouth bass	0.979 (2.273)	0.948 (1.812)	0.973 (2.146)	0.902 (1.483)
White bass	0.943 (1.764)	0.860 (1.293)	0.909 (1.522)	0.773 (1.028)
Warmouth	0.954 (1.874)	0.856 (1.278)	0.921 (1.596)	0.641 (0.760)
Freshwater drum	0.863 (1.305)	0.703 (0.873)	0.934 (1.689)	0.747 (0.966)

TABLE 5. Analysis of variance of the means of the correlation coefficients obtained by two observers using four different aging methods (Scales, vertebrae, otoliths and operculars). Data transformed by z-transformation procedures.

Source of variation	Sum of squares	df	MS	F
Between methods	2.282	3	0.7606	5.870*
Within methods	4.146	32	0.1296	
Total	6.428	35		

* 0.05 level

TABLE 6. Results of a simultaneous sums of squares analysis for differences in the mean z-transformation of correlation coefficients for four methods of aging scaled fishes (r has been converted from the mean z-transformation to the mean correlation coefficient).

Method	Operculars	Vertebrae	Otoliths	Scales
Mean r	0.763	0.821	0.916	0.919

Catfishes

Mean ages obtained for catfishes by each observer were similar no matter what method of aging was used (Tables 7 and 8). The analysis of variance of the ranked mean ages obtained from the three aging methods showed no significant statistical difference between methods or observers (Table 8).

Correlation coefficients and their corresponding z-transformations for estimated ages obtained by the two observers are shown in Table 9. Results of the analysis of variance of z-transformations indicated no significant statistical difference existed between the correlations obtained by the three methods of aging catfishes (Table 10). Any of these three methods of aging catfishes should result in correlation coefficients between 0.825 and 0.922 95 per cent of the time.

DISCUSSION AND CONCLUSIONS

Although the relationship between the estimated ages and the true ages of fishes investigated in this study is unknown, conclusions can be drawn regarding the consistency of the applied methods.

Scales and otoliths are regarded as the preferred methods for aging scaled fishes because of their high correlation coefficients. Aging scaled fishes by opercular bones is the least preferred method because of the poor agreement of ages of fishes obtained between readers. Aging scaled fishes by vertebrae appears to be intermediate to aging by scales, otoliths and opercular bones.

Aging scaled fishes by their scales results in higher age estimates than any of the other methods used in this study. Aging fishes by their vertebrae gives lower age estimates than other aging methods.

No significant statistical differences were found between correlation coefficients obtained by aging catfishes from vertebrae, opercular bones, or pectoral spines. No significant difference was found in the ages reported for each aging method. Therefore, none of the methods would be preferred over the other as far as reading year marks is concerned.

In order to evaluate the validity of the aging methods used in this study, further investigation is needed. The relationship between known age fishes and their predicted age must be known before any method of aging is adopted for Texas waters. Validity of annuli on scales as year marks can be obtained from age and growth data itself. For example, annuli may be regarded as year marks if there is a proper correlation between the presumable age of a fish and its size, there are agreements among calculated growth histories; and there is a persistent abundance or scarcity of certain year-classes in collections over several years. An effort to apply these tests was made in this study, but with little success. So, without knowing the relationship between the true age of a fish and the age predicted by the methods used in this study, it is not possible to make a recommendation of the best method to use. A study with known age fishes will permit such a recommendation to be made.

TABLE 7. Mean ages (\pm standard error of the mean) obtained by two observers aging the same set of catfish by three different methods.

Species	Method ¹	n	Mean first observer	Mean second observer
Blue catfish	2	63	2.32 \pm 0.35	2.25 \pm 0.33
	4	64	2.25 \pm 0.26	2.31 \pm 0.29
	5	60	2.07 \pm 0.27	2.10 \pm 0.27
Channel catfish	2	137	1.59 \pm 0.17	1.61 \pm 0.17
	4	117	1.80 \pm 0.14	1.86 \pm 0.14
	5	134	1.75 \pm 0.17	1.78 \pm 0.17
Flathead catfish	2	18	3.56 \pm 0.87	3.83 \pm 0.84
	4	17	3.41 \pm 0.57	3.71 \pm 0.56
	5	17	3.23 \pm 0.90	3.35 \pm 0.94

¹ Method: 2 = vertebrae; 4 = opercular bones; 5 = pectoral spines.

TABLE 8. Results of analysis of variance for coded mean ages obtained by each observer using three different methods of aging catfishes (vertebrae, opercular bones, and pectorial spines).

Source of variation	Sum of squares	df	MS	F
Method	0.00001	2	0.000005	0.00001
Observer	0.33730	1	0.337300	1.05160
Interaction	0.11480	2	0.057400	0.17890
Error	3.84890	12	0.320700	
Total	4.30110			

* 0.05 level

TABLE 9. Correlation coefficients obtained for two observers aging the same set of catfish, () equals the z-transformation of the correlation coefficient.

Species	Technique		
	Vertebrae	Operculars	Spines
Blue catfish	0.793 (1.070)	0.858 (1.285)	0.882 (1.386)
Channel catfish	0.930 (1.658)	0.775 (1.033)	0.927 (1.637)
Flathead catfish	0.933 (1.681)	0.782 (1.050)	0.933 (1.681)

Table 10. Analysis of variance of z-transformations of the means of the correlation coefficients between two different observers aging the same set of catfishes by three different methods (vertebrae, opercular bones, and pectoral spines).

Source of variation	Sums of squares	df	MS	F
Between methods	0.328	2	0.1642	2.983
Within methods	0.330	6	0.0550	
Total	0.659	8		

* $0.05 < P$

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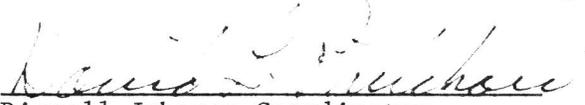
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