

Age Estimation and Length Back-calculation for Known-age Largemouth Bass

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Abstract: Scales and otoliths from largemouth bass (*Micropterus salmoides*) of known ages (age 6, 7, and 8) were prepared and examined by 3 experienced readers who were uninformed of specimen age or length. Results indicated limited agreement between readers and numerous inaccurate age estimations. Among 97 scale samples read, all 3 readers only agreed on the age of a single specimen which was older than estimated; using otoliths, readers agreed on 4 specimens which were correctly aged. Percentage of correctly-aged fish ranged from 10% to 30% with scales and 39% to 47% with otoliths. Percent of fish correctly aged ± 1 year ranged from 29% to 77% with scales and 78% to 98% with otoliths. Back-calculated lengths-at-age based on scale annulus measurements made by a fourth reader who knew specimen age, length, and sex were typically less than actual measurements taken each year (in October) with the greatest differences for earlier years of life. Therefore, accurately aged scales provided back-calculated lengths as expected for criteria in this study. Similar back-calculations based on otolith annuli yielded inconsistent results without obviously greater deviations from known length in younger fish and lesser deviations in older fish.

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Largemouth bass is one of the most important freshwater game fishes in Texas. Consequently, effective management of this species has been a major concern of the Texas Parks and Wildlife Department (TPWD).

Critical elements of largemouth bass management include the ability to accurately age specimens and use annular marks to back-calculate length-at-age (Carlander 1973). Inaccuracies in efforts to age fish or in back-calculated lengths has lead to errors in both management practices and basic understanding of fish populations (Beamish and McFarlane 1983). Numerous studies have exam-

ined age-estimation differences between scales and otoliths, or other hard parts, and back-calculated lengths generated from those data. Jearld (1983) and others stressed the need for validation of age estimates. Beamish and McFarlane (1983) argued that age validation can only be achieved through the use of mark-recapture techniques or known-age fish.

Several papers have discussed efforts to use known-age largemouth bass to verify annular marks. Scales were used for age 2+ (Prather 1966) and age 4+ fish (Prentice and Whiteside 1974). Otoliths were used to age 0+ (Isley and Noble 1987, Isley et al. 1987), age 4+ (Taubert and Tranquilli 1982, Perry and Tranquilli 1984, Maceina and Murphy 1989), and age 5+ (Hoyer et al. 1985) specimens. In general, these efforts utilized a known stocking date and subsequent collection of specimens to validate age. No published studies appear to have utilized known-age largemouth bass older than age 5 and actual lengths by year for individual fish were unavailable. Because largemouth bass management strategies often focus on large or trophy specimens, correctly aging larger and older fish can be important.

A study of largemouth bass growth rates started at TPWD's Heart of the Hills Research Station (HOH) in 1985 included more specimens than needed for that investigation. Subsequently, for this study a group of largemouth bass with known ages (ages 6 through 8) and known growth history became available for validation of scale- and otolith-age estimates and examination of back-calculated length-at-age estimates.

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Methods

In May 1985, a pair of largemouth bass spawned in a 0.7-ha pond at HOH, Kerr County, Texas. Fry were placed into a separate pond that had been fertilized and stocked with a variety of small forage fishes. In October 1985, this pond was drained and juveniles were recovered. From these, 400 of the smallest and 400 of the largest specimens were selected and subsequently restocked in separate research ponds. These test fish were maintained in separate ponds from 1985 to 1990, then combined in the same pond from 1990 through 1993. Each October, ponds were drained and all bass were counted and measured (nearest mm total length, TL) before being restocked into research ponds. Fish never spent 2 consecutive years in the same pond. Pond sizes ranged from 0.7 to 1.0 ha. Ponds were stocked with a variety of forage fishes including gizzard shad (*Dorosoma cepedianum*), fathead minnow (*Pimephales promelas*), golden shiner (*Notemigonus crysoleucas*), mosquitofish (*Gambusia affinis*), green sunfish (*Lepomis cyanellus*), and goldfish (*Carassius auratus*). Size and number of forage fish provided was increased as bass grew. Additionally, during each October pond

draining, any young-of-the-year bass produced by the test fish were removed. Mean monthly minimum and maximum air temperatures for the study area were obtained from the Office of the State Climatologist, College Station, Texas, covering the period May 1985 through December 1992.

In October 1988 (at age 3), fish were injected with passive integrated transponder (PIT) tags (Northwest Mar. Tech., Seattle, Wash.) as described by Prentice et al. (1991). A malfunction in the PIT tag reader prevented obtaining PIT tag numbers for some specimens until October 1989. During all subsequent years, individual lengths were associated with a PIT tag number for each fish. In October 1991 (at age 6), a group of 50 bass was randomly selected from the remaining fish; each was measured, scale and otolith samples were removed, and PIT tag number was recorded. Another group of 25 fish was sampled in 1992 (at age 7) and a final group of 25 fish was similarly taken in 1993 (at age 8).

Scales were cleaned in a weak detergent and water solution and mounted between 2 microscope slides. One otolith from each fish was fixed to an acetate slide with epoxy cement, then ground with a belt sander by methods similar to those described by Maceina (1988). Otolith sections were made in the transverse plane as close as possible to the core or nucleus. Age-assignment techniques followed those of Prentice and Dean (1991). Scales were examined on an Eberback Corporation scale reader. Scale annuli were counted and measured along an axis from the focus diagonally to the margin (greatest possible radius). Otoliths were examined with transmitted light under a microscope with an optical micrometer. Otolith age was estimated along any axis, but otolith measurements were taken only from the core to the dorsal margin along the longest axis (several specimens were aged but annuli could not be measured along the designated axis). Readers were instructed to regard samples as they would field-collected scales and otoliths in normal day-to-day work. Readers were also instructed to omit any sample they considered unreadable. All readers used the same equipment at the same magnifications for all scale and otolith samples. Test fish from which both scales and otoliths were unreadable were excluded ($N = 3$). A total of 97 specimens was ultimately used.

Each scale and otolith sample was aged and measured independently by 4 readers including fishery management and research biologists with 9–25 years of experience aging fish and making annular measurements. Samples were coded and order was mixed. Readers 1–3 aged and measured annuli on scales and otoliths without prior knowledge (termed naive readers) of specimen age, length, or sex. Naive-reader data were used to assess accuracy of age estimations. Reader 4 knew age, length, and sex of each specimen and attempted to designate appropriate annuli for each scale and otolith for use in back-calculated length-at-age comparisons.

We assessed the accuracy of scale and otolith ages from the 3 naive readers by examining mean difference from known ages and percent agreement with known ages (Welch et al. 1993). Precision of scale and otolith ages was assessed by calculating mean coefficient of variation (Chang 1982) of the 3 age estimates for each fish and averaging these for the entire sample.

Using the direct proportion back-calculation method, we estimated lengths of fish back to age 3. This age corresponds to the year that fish were individually marked with PIT tags. Back-calculated lengths estimate fish size at the time of annulus formation, usually spring in the southern United States (Jearld 1983). Our observed lengths were taken in October, after the summer growing season. We computed the difference between back-calculated lengths and observed lengths and looked for differences in patterns derived from otoliths and from scales. If scales and otoliths accurately reflect changes in body size, the magnitude and pattern of differences between back-calculated and observed lengths should be similar for both structures. Also, we established 2 criteria to determine if back-calculations based on scales or otoliths were acceptable: 1) negative values for differences between back-calculated and observed lengths (this would be expected as a result of summer growth), and 2) similarity between annual length, derived from back-calculations, and observed length in October.

Results

Temperatures and Fish Growth

Mean monthly minimum and maximum air temperatures (C) were 0.7 and 15.1 for January and 20.2 and 33.5 for August, respectively (Fig. 1). Mean observed total length (mm) for sexes combined was 155, 284, and 299, respectively, from age 0 through age 2. Mean observed total length for males was 331, 355, 383, 411, 424, and 418 from age 3 through age 8. Mean observed total length for females was 353, 391, 428, 472, 497, and 512 for the same age groups.

Comparison of Age Estimates from Naive Readers

Age estimates were obtained by 2 naive readers for all 97 scale samples and by the third naive reader for 96 scale samples. However, among otolith samples, all 3 readers considered 2 unreadable and 9 others were rejected by at least 1 reader. Mean age estimates from scales for age 6 and 7 fish were high from

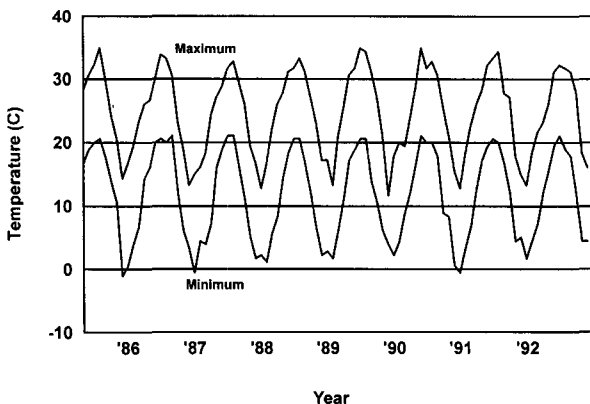


Figure 1. Mean monthly minimum and maximum air temperatures for the Kerr County, Texas, area, May 1985 through December 1992, obtained from the Office of State Climatologist, College Station, Texas.

1 reader but low from the other 2 readers while those for age 8 fish were low from all 3 readers (Table 1). Mean age estimates from otoliths for age 6 fish were high from 2 readers, but low from 1, while for age 7 and 8 fish, were low from 2 readers and slightly high from the third (Table 1).

Using scales, readers agreed on the age of a single fish which was underestimated by all 3 readers. With otoliths, all 3 readers agreed on 4 fish, all of which were assigned the correct age. No agreement between all 3 readers and both body parts occurred. The frequency of correctly-aged fish for Readers 1, 2, and 3 was 30%, 8%, and 10%, respectively, using scales and 43%, 47%, and 39%, respectively, using otoliths (Table 2). However, a difference of only 1 year or less between estimated and known ages ranged from 29% to 77% with scales and 73% to 98% with otoliths (Table 2). Mean coefficient of variation of age estimates among readers was 29.7% with scales and 13.7% with otoliths.

Back-calculated Length-At-Age

Back-calculated lengths from scale annulus measurements produced mean lengths (at the time of annulus formation) less than known lengths (October) for all fish combined (Table 3); those fish terminated at age 6, 7, and 8 (Table 3); and males and females (Table 4). The magnitude of the difference was greatest for ages 3 and 4 and decreased for annular measurements taken progressively nearer the scale margin.

Back-calculated lengths generated from otolith annulus measurements typically produced estimates greater than known lengths for the inner-most annuli and less than known lengths for outer annuli for all fish combined (Table 5), ages 6 and 8 (Table 5), and females (Table 6). Back-calculated lengths were less than measured known-October lengths for age 7 fish (Table 5) and males (Table 6). The pattern of greater deviation with measurements from inner annuli and lesser deviation from outer annuli, seen in scales, was not evident in otoliths.

Table 1. Mean estimated age and SE determined by 3 naive readers from scales and otoliths removed from known-age largemouth bass ages 6–8 reared at Heart of the Hills Research Station, Kerr County, Texas, 1985–1993.

Known age	Reader 1		Reader 2		Reader 3	
	Mean age estimated	SE	Mean age estimated	SE	Mean age estimated	SE
			Scales			
6	7.1	0.16	4.2	0.15	4.5	0.14
7	7.7	0.20	4.7	0.17	5.0	0.15
8	7.9	0.13	5.8	0.26	5.5	0.17
			Otoliths			
6	6.8	0.07	5.3	0.11	6.8	0.24
7	7.3	0.10	6.3	0.25	6.2	0.24
8	8.4	0.10	7.4	0.16	8.2	0.26

Table 2. Frequency and percent agreement of estimated ages determined by 3 naive readers from scales and otoliths of largemouth bass of known ages (ages 6–8) reared at Heart of the Hills Research Station, Kerr County, Texas, 1985–1993.

Scales			Otoliths		
Age difference	Frequency	Percent	Age difference	Frequency	Percent
Reader 1					
0	29	30.2	0	39	42.9
1	45	77.1	1	50	97.8
2	17	94.8	2	2	100.0
3	5	100.0			
Reader 2					
0	8	8.2	0	42	47.2
1	20	28.8	1	34	85.4
2	34	63.9	2	12	98.9
3	29	93.8	5	1	100.0
4	6	100.0			
Reader 3					
0	10	10.3	0	35	39.3
1	20	30.9	1	30	73.0
2	43	75.2	2	17	92.1
3	20	95.8	3	4	96.6
4	4	100.0	4	2	98.8
			6	1	100.0

Discussion

Age Estimation

Otoliths provided age estimation accuracy higher than scales, but lower than expected. Also, scales tended to underestimate true age compared to otoliths. Differences between scale and otolith age estimates, as found in this study, have been reported by other researchers. Age estimates for a coregonid were reported by Skrudal et al. (1985) as lower from scale estimates than from otoliths; similar findings were reported by Prentice and Dean (1991) for a sciaenid and Erickson (1983) for a percid. Welch et al. (1993) found age estimates from scales, spines, and rays to be generally within 1 year from striped bass (*Morone saxatilis*) up to 900 mm TL (age 10), but these structures provided increased underestimation with increase in age and greater variation at all ages compared to otoliths. However, Maraldo and MacCrimmon (1979) found good statistical agreement for age estimates in largemouth bass from northern latitudes with scales and otoliths, but scales were more likely to underestimate true ages.

Explanations for aging errors could fall into 3 considerations: 1) structures not displaying proper marks to represent, or be interpreted as representing, the proper age; 2) a technique to prepare a structure for interpretive viewing does not allow proper visualization of marks; or 3) variations in reader interpretation. The annual pond draining and fish handling in this study may have de-

Table 3. Mean of back-calculated minus actual length-at-age (mm total length) from scale annuli determined by a reader who knew specimen age for largemouth bass reared at Heart of the Hills Research Station, Kerr County, Texas, 1985–1993.

Group	Annulus age	N	Mean	SE
All fish combined	3	44	-64.0	6.29
	4	97	-51.6	4.34
	5	97	-39.8	3.98
	6	97	-25.0	2.51
	7	48	-22.4	2.17
Age 6	8	25	-12.6	1.60
	3	26	-43.5	6.70
	4	49	-28.3	5.10
	5	49	-17.2	4.87
Age 7	6	49	-16.9	2.23
	3	9	-72.4	7.27
	4	23	-61.0	4.29
	5	23	-48.6	3.11
Age 8	6	23	-18.4	2.36
	7	23	-17.5	1.74
	3	9	-144.8	9.89
	4	25	-88.4	8.43
	5	25	-76.0	6.69
	6	25	-47.1	6.77
	7	25	-26.9	3.65
	8	25	-12.6	1.60

creased clarity of both scale and otolith growth markings, but no unhandled fish were available for comparison.

We assumed through experience and review of past studies that otoliths and scales prepared as described would properly show age markings which might be present. Some studies have suggested sectioned otoliths produce greater error than whole otoliths (Hoyer et al. 1985, Maceina and Murphy 1989), but they did not examine specimens as old as those here. Most otoliths from fish in this study were thick and opaque, and required sectioning to see all but the outer-most annuli. However, there is the possibility that different preparations might have more clearly shown age marks and increased aging accuracy in this study.

Many studies employ 2 or 3 independent readers and require agreement among all before assigning an age, agreement with a second reading, discussion among readers to agree upon an age, or rejection of the sample for non-agreement. In some cases, paired sets of scales and otoliths are examined simultaneously to obtain an age estimate. Prentice and Whiteside (1974) correctly aged 84%-96% of known-age largemouth bass age 2 through age 4 using scales.

Table 4. Mean of back-calculated minus actual length-at-age (mm total length) from scale annuli determined by a reader who knew specimen age for largemouth bass reared at Heart of the Hills Research Station, Kerr County, Texas, 1985–1993.

Annulus age	N	Mean	SE
Males			
3	14	-70.8	9.64
4	26	-51.3	5.54
5	26	-46.2	5.17
6	26	-24.1	4.85
7	11	-22.7	4.49
8	3	-15.8	6.13
Females			
3	30	-60.8	8.09
4	71	-61.7	5.60
5	71	-37.5	5.08
6	71	-25.3	2.95
7	37	-22.3	2.51
8	22	-12.1	1.66

However, they required agreement with subsequent rereadings (66% of their scales had to be read a second time). Similarly, Prather (1966) reported correct age estimations in 80% of largemouth bass age 0+ through age 2+ and increasing accuracy to 84% upon rereading. Fish in the present study were older and age determination may become increasingly difficult in older, larger fish (Beamish and McFarlane 1987). Readers in this study did not confer following their initial reading, reread samples, or examine scales and otoliths side by side. Logically, discussion, reexamination, or comparison of structures may have increased age estimation accuracy and agreement among readers.

Most largemouth bass collected in Texas waters and aged by fishery management biologists are estimated to be age 0 to age 5 (TPWD, unpubl. data). If ages are correct, older specimens are rarely encountered. Indeed, at the onset of this study in 1985, only a single largemouth bass taken in Texas was aged older than 7 years (A. A. Forshage, TPWD, Tyler, pers. commun.); older largemouth bass have been reported since that time. Among the readers in the present study were 1 research biologist and 2 management biologists. The majority of management biologists' day-to-day aging experience was with young fish obtained from routine population survey sampling. The 2 readers routinely seeing young fish were more inclined than the third reader to underestimate age with both scales and otoliths. However, even with the possible bias of readers toward underestimated aging, the degree of agreement between all readers at the age

Table 5. Mean of back-calculated minus actual length-at-age (mm total length) from otolith annuli determined by a reader who knew specimen age for largemouth bass reared at Heart of the Hills Research Station, Kerr County, Texas, 1985–1993.

Group	Annulus age	N	Mean	SE
All fish combined	3	33	15.3	7.51
	4	75	10.6	4.05
	5	75	-1.2	4.23
	6	75	-20.9	2.00
	7	38	-23.4	2.64
Age 6	8	21	-20.8	2.35
	3	21	16.5	9.39
	4	37	16.6	5.91
	5	37	13.2	6.72
Age 7	6	37	-18.1	2.91
	3	4	-20.6	23.26
	4	17	-5.5	7.83
	5	17	-19.3	5.69
	6	17	-22.9	3.33
Age 8	7	17	-23.3	2.41
	3	8	30.0	12.69
	4	21	13.2	7.23
	5	21	-11.9	5.86
	6	21	-24.1	4.17
	7	21	-23.5	4.42
	8	21	-20.8	2.35

± 1 -year accuracy level indicates otoliths can be used in aging older largemouth bass. If management regulations and strategies, such as restrictive size and bag limits and catch-and-release practices, ultimately increase largemouth bass survival, older fish in samples may become more common in the future. Periodic training and experience with aging older fish should aid in reducing variation in reader age interpretations.

In spite of the absence of rereading to assist age assignment, the selection of a single structure preparation technique each for scales and otoliths, and the apparent variations in reader age interpretations, otoliths provided ages from all readers in this study that were more accurate than scales and that can be expected to provide age estimates within ± 1 year of true ages. Managers should use otoliths to age older largemouth bass and be aware of the possibility of a 1-year age error.

Back-calculated Length-at-Age

Back-calculated body lengths derived from scale annulus measurements were consistent across ages and sexes and with study criteria, providing decreasing negative differences between back-calculated and observed lengths. Expected decreases in growth increments were seen in the observed fish growth

Table 6. Mean of back-calculated minus actual length-at-age (mm total length) from otolith annuli determined by a reader who knew specimen age for largemouth bass reared at Heart of the Hills Research Station, Kerr County, Texas, 1985–1993.

Annulus age	<i>N</i>	Mean	SE
Males			
3	11	-17.4	11.22
4	20	-13.4	7.07
5	20	-16.5	9.10
6	20	-26.3	4.69
7	9	-28.7	4.95
8	3	-17.5	6.10
Females			
3	22	31.6	7.79
4	55	19.4	4.17
5	55	4.4	4.54
6	55	-18.9	2.09
7	29	-21.8	3.08
8	18	-21.3	2.60

which supports the study criteria. Back-calculated body lengths derived from otolith annulus measurements were not consistent across ages or sexes or with study criteria. Although other authors have reported good relationships between otolith annulus measurements and actual lengths-at-age, these studies utilized younger fish than used in this study. For example, largemouth bass to age 5 (Hoyer et al. 1985) and age 4 (Perry and Tranquilli 1984) were used to back-calculate body lengths from otoliths. Otoliths from those studies did have inner annuli with decreasing increments for the first few years, but thereafter, annuli were often near equally spaced. Because back-calculated lengths from otoliths in this study were inconsistently different from lengths-at-age measured each October, we suspect there are problems using otoliths for back-calculations in older largemouth bass following the techniques described in this study. Back-calculated lengths generated from correctly-aged scales appeared to reflect observed length-at-age and annual increase in length of older largemouth bass. The problem with scales in this study was the apparent lack of ability of managers to obtain correct ages. Additional work in back-calculating lengths for older largemouth bass is needed to define usable techniques.

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