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Comparison of Aging Methods and Validation of Otolith Ages for the Rainbow Darter, *Etheostoma caeruleum*

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***Etheostoma caeruleum* were sampled from the James River, southwest Missouri, during eight months, July 1996 through May 1997. Ages were estimated from whole and sectioned otoliths (sagittae) and from scales by counting annuli. Edge analysis indicated that otolith annuli (opaque zones), visible in whole and cross-sectioned otoliths, formed yearly, primarily during July through September. In contrast, scale edge analysis indicated no clear pattern of annulus formation. Independent readers agreed on 63–73% of ages using otoliths but only 38% of ages using scales. Sectioned and whole otolith age estimates made by one experienced reader agreed for 68% of individuals. Scales underestimated age compared to otoliths, and maximum otolith age was five years.**

THERE has been an increased effort to validate (estimate the accuracy of) age estimates from hard parts of fishes since Beamish and McFarlane's (1983) review (e.g., Barger, 1985; Schramm, 1989; Beckman et al., 1991). However, age estimates made by counting zones in hardparts have been validated for few freshwater fishes other than common game fish species (Taubert and Tranquilli, 1982; Maceina and Betsill, 1987; Schramm, 1989). Age estimations are commonly reported for darters of the genus *Etheostoma* (e.g., Wolfe et al., 1978; James and Taber, 1986; Layman 1991). However, using the validation criteria suggested by Beamish and McFarlane (1983) and defined by Kalish (1995) as ''determining the temporal meaning of the zones being counted,'' there have been few reported attempts to validate age estimates for species of darters.

The majority of age estimations for *Etheostoma* species have been performed by counting scale annuli (e.g., Wolfe et al., 1978; James and Taber, 1986), often supplemented by length-frequency analysis (e.g., Fisher, 1990; Knight and Ross, 1992; Layman, 1993). Although length-frequency analysis can be quick and simple to apply, it is an imprecise method of age estimation for fishes such as darters (Taber and Taber, 1983; James and Taber, 1986), hampered by variable growth among individuals in an age class and slowing or stoppage of growth in oldest age classes (MacDonald, 1987). Scale age estimation methods are nonlethal but are often times difficult to validate or apply with precision (Beamish and McFarlane, 1987). Underestimation of age is common using scales (Beamish and McFarlane, 1983; Casselman, 1987) and few studies have reported validated scale aging methods for *Etheostoma* (Fahy, 1954; Johnson and Hatch, 1991).

Otoliths provide an alternative to traditional aging methods and have proven to be more reliable for most species when compared to other aging structures (e.g., Beamish and McFarlane, 1983; Sikstrom, 1983; O'Gorman et al., 1987). Yearly age estimates have not been reported using otoliths for *Etheostoma* species, possibly because it requires lethal sampling and is typically more time-consuming than scale aging. Method comparisons assist in evaluating which method is most efficient, reliable, and appropriate for a given aging study.

Validation can be most easily achieved if known-age individuals are available. However known-age individuals are rarely available in wild populations. Hardpart edge analysis provides an alternative, indirect method of validation that has been applied successfully for many fish species (Beckman and Wilson, 1995). This technique involves following the progression of annulus formation at the growing edge of the hardpart in individuals taken from a population throughout the year to determine whether one annulus is formed per year. The purposes of this study were (1) to determine whether one annulus was formed per year in the scales and otoliths of rainbow darters, *Etheostoma caeruleum*, and thus validate age estimates, and (2) to evaluate the precision of age estimates by comparing estimates made using various methods by three separate readers.

Materials and Methods

Rainbow darters were collected from the James River of the White River system in southwest Missouri, in each of eight months from July 1996 through May 1997. Samples were taken from various riffles within a section of the river about 3.5 km long located east of Springfield, Missouri. Monthly samples of 10–18 rainbow darters were taken by hand-held seines and sweep nets, after passing through the area with a backpack electrofisher. After capture, fish were placed on ice and returned to the laboratory and preserved in 95% ethanol.

Otoliths (sagittae) and scales were removed from preserved specimens. One otolith from each fish was mounted whole to a microscope slide with Permount mounting medium such that it could be viewed through the sagittal plane. The other otolith was embedded in Embed 812 epoxy resin and sectioned with a low-speed saw. A 0.5 to 1 mm thick section was sanded with 600 grit sandpaper and polished with 0.3 micron alumina micropolish (Thompson and Beckman, 1995). About three scales were removed from each of three separate body regions of each fish: (1) nape, (2) just anterior to the lateral line at midbody, and (3) caudal peduncle. These regions were chosen to assure that the most readable scales would be available for age estimation (Jearld, 1983). Scales from all body regions were mounted together between two glass slides for viewing (Karr, 1963).

Annuli were counted on otoliths and scales using transmitted light and either a dissecting microscope (7x to 40x) or a compound microscope (40x or 100x). The number of annuli and edge conditions were assigned without the knowledge of fish size or the sample date. Three readers counted annuli and evaluated the growing edge (margin) of each structure for each individual fish (with the exception of scales, for which Reader 3 estimates were unavailable). Readers 1,2, and 3 had approximately >10, 2, and 0 years, respectively, of experience aging fish using hardparts.

Otolith annuli were defined as distinct narrow zones of growth that were opaque relative to surrounding areas. These annuli reflect seasonal differences in the protein:calcium ratio in the fish otolith and are likely formed as a physiological response to environmental variation (e.g., temperature change) or in correspondence with variations in somatic growth (Casselman, 1990; Beckman and Wilson, 1995; Fowler, 1995). Otolith margins were classified as opaque if an annulus was observed at the growing edge and translucent otherwise. Each reader selected one scale that was judged to exhibit the clearest growth pattern. Scale annuli were defined as closely spaced circuli, cutting over of circuli, and opaque regions (Lachner et al., 1950; Fahy, 1954; Karr, 1964). Closely spaced circuli are typically laid down in fish scales during the cold months in correspondence with slow body growth. These closely spaced circuli may appear as an opaque region relative to the surrounding regions. Cutting over occurs when a complete circulus, formed during a fast growth period, intersects with an incomplete circulus, formed during a prior slow growth period (Jearld, 1983).

To determine in which season annuli were formed and whether one annulus was formed each year, I followed the growth of the scale and otolith edges in the population through one year. Edge values were assigned based on the amount of growth on scales and otoliths beyond the last complete annulus. Values of +, ++, and +++ were used to indicate increasing amounts of scale growth, with + indicating an annulus forming at the margin, ++ indicating substantial scale growth beyond the last annulus formed, and +++ indicating a scale apparently near the formation of the next annulus (based on spacing of other annuli). The percent of individuals with each otolith and scale edge condition was calculated by month. A single peak would be expected if an annulus is formed concurrently in individuals in the population.

A precise estimate of age was assigned using the time of annulus formation and edge values, along with an estimate of the time of spawning. The use of annulus and edge data allows the assignment of a more accurate age than using annulus counts alone (Casselman, 1987). It avoids assigning different ages to fish that were actually born during the same year, which can occur when fishes of the same year class do not form an annulus at precisely the same time (Beckman and Wilson, 1995). For example, the margin data allow for the assignment of an extra year to the age of a fish assigned a +++ edge condition if it is near the time of annulus formation (Beckman et al., 1991). Assigning a realistic birth date avoids the bias that is introduced into growth data by assuming an arbitrary birthday (e.g., January 1). This is especially important for short-lived fishes, for which a significant amount of growth can occur over a fraction of a year.

Number of annuli and edge conditions were assigned independently from scales, whole otoliths, and sectioned otoliths. A birth date of 1 April, the middle of the reported spawning period in Missouri (Pflieger, 1997), was assumed for assigning ages. Variability among age estimates made by three independent readers was characterized by percent agreement, mean difference, and the coefficient of variation (Chang, 1982). The effect of variability among aging methods was illustrated by a comparison of age frequency as determined by the three methods.

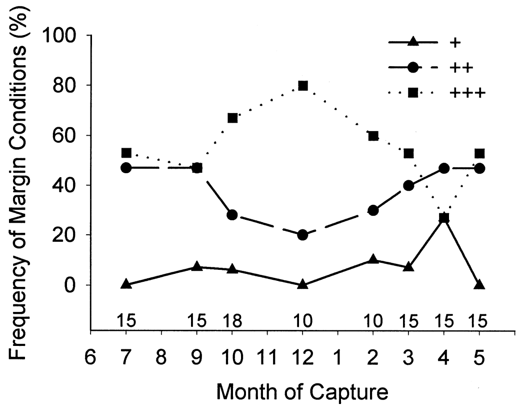

Fig. 1. Relationship of observed frequency of occurence of three margin conditions in scales of *Etheostoma caeruleum* versus month of capture, 1996–1997. Margin condition was based on the estimated degree of completion of the annulus at the growing edge of the scale: (+) annulus forming at the margin, (++) substantial scale growth beyond the last annulus, (+++) scale apparently near the formation of the next annulus. Numbers above the x-axis indicate sample sizes.

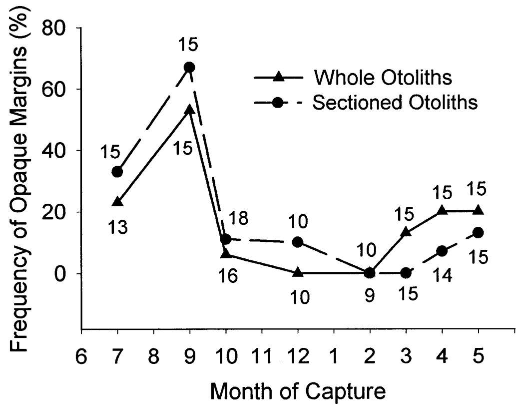

Fig. 2. Relationship of observed frequency of occurrence of opaque zones (annuli) at the growing margin in whole and sectioned otoliths (sagittae) of *Etheostoma caeruleum* by month of capture, 1996–1997. Numbers next to the points indicate sample sizes. Differences in sample sizes are caused by samples lost or damaged during sectioning.

Results

Discernable annuli were formed less frequently in scales than in otoliths. Although the time needed to count annuli and assess edge conditions was not monitored, readers agreed that more time was spent on scales because the annuli were not as well defined. The otoliths (sagittae) of rainbow darters are flattened and translucent, so that annuli (opaque zones) were visible in whole otoliths viewed through the sagittal plane. However, annuli were more distinct in transverse sections than in whole otoliths.

The plot of scale margin conditions (Fig. 1) does not indicate a single time period in which annuli are formed. Although a mode in +++ values is indicated in December, the majority of scales in most months were classified with a +++ edge condition.

Plots of otolith margin condition by month (Fig. 2) show a clearly defined peak for otoliths viewed either whole or in cross-section. This suggests that the majority of rainbow darters formed one annulus in their otoliths each year some time during the months of July, August, or September. Thus counts of annuli on the otolith can be used to determine age of rainbow darters in years.

Otolith age estimates were in agreement for greater than 60% of all pairwise comparisons among readers (Table 1). The mean difference in age estimates between readers was approximately 0.2 years or less for all comparisons. Between reader otolith age comparisons that in-

Table 1. Comparison of Age Estimates (Years) of *Etheostoma caeruleum* Made by Three Readers Using Three Aging Methods. Readers experience indicated as (1) most, (2) intermediate, and (3) least experienced. Numbers separated by colons indicate pairwise reader comparisons. Percent agreement is the percent of fish for which age estimates were identical. CV = coefficient of variation.

	Percent agreement (*n*, mean difference) Reader comparisons			
Aging method	1:2	1:3	2:3	CV
Sectioned otoliths	73%	69%	64%	0.161
	(112, 0.09)	112, −0.13)	(113, 0.21)	
Whole otoliths	65%	63%	63%	0.179
	(108, −0.06)	(10, 0.06)	(106, 0.12)	
Scales	38%			0.348
	(112, −0.59)			

TABLE 2. PAIRWISE COMPARISONS OF AGE ESTIMATES OF *Etheostoma caeruleum* MADE BY AN EXPERIENCED READER USING THREE AGING METHODS. SO = sectioned otoliths, WO = whole otoliths, Sc = scales. Percent agreement is the percent of fish for which age estimates were identical. CV = coefficient of variation.

Aging methods	SO:WO	SO:Sc	WO:Sc
Percent agreement	68%	38%	40%
Mean age difference	0.03	0.71	0.66
CV	0.169	0.277	0.282
n	107	112	108

cluded the most experienced reader showed the best agreement. Values for the coefficient of variation indicate low variability among the three readers' age estimates for both whole and sectioned otoliths. Scale age estimates by the two most experienced readers were more variable and showed lower agreement than any of the otolith age comparisons. These data suggest that whole and sectioned otoliths yield more precise age estimates than scales. Comparison of the precision of the three aging methods made by the most experienced reader (Reader 1) show high agreement and low variability for comparisons between sectioned and whole otolith age estimates, and low agreement and higher variability between otolith and scale age estimates (Table 2).

Age distributions based on sectioned and whole otoliths (Fig. 3) are not significantly different (Kolmogorov-Smirnov 2-sample test; $P = 0.961$). Scale-based age distributions, however, were significantly different from sectioned and whole otolith age distributions ($P < 0.0001$). If scale ages are used, the maximum age indicated is three years and the majority of individuals would be classified as two years of age. Age distributions based on the validated otolith data (Fig. 3) indicate a maximum age of five years, with the majority of individuals at two years age, with relatively low numbers of zero- and one-year olds in our collection. Maximum size of fish collected was 67 mm total length.

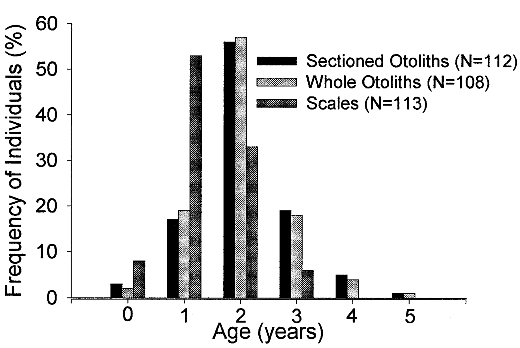

Fig. 3. Frequency distribution of age estimates for *Etheostoma caeruleum* using three aging methods.

DISCUSSION

The absence of an annulus at the growing edge of scales during a single time period suggest that rainbow darters do not form a single annulus in their scales each year. Difficulty in determining scale annuli has also been reported for other darter species (e.g., Karr, 1963, 1964; Taber et al., 1986). Data supporting the yearly formation of scale annuli has been reported for some species of darters (e.g., Fahy, 1954; Hill, 1968); however, one cannot take this as validation of scale age estimation for other species of darters (Beamish and McFarlane, 1983). If precise age estimates are desired, scales are not recommended for rainbow darter age determination. In this study, readers were unable to obtain valid or precise ages using scales.

The concurrent formation of the otolith opaque zone in rainbow darters from this population in summer months supports the hypothesis that the opaque zone forms as a response to increases in growth rate (Beckman and Wilson 1995), because the peak feeding intensity of the rainbow darter is during early summer (Etnier and Starnes, 1993). However, opaque zone formation could also be a response to changes in temperature or other physical variables during summer months. Timing of opaque zone formation matched that observed for white sucker *Catostomus commersoni* (Thompson and Beckman, 1995) from the same geographic region.

The formation of one annulus per year indicates that rainbow darter otoliths can be used to reliably determine age. High agreement among readers of varying experience on ages in whole and sectioned otoliths suggests that whole otoliths produce reliable age estimates and the extra time and expense involved in sectioning otoliths can be avoided.

The maximum age of five years for rainbow darters from the James River is greater than that reported for darters in other studies, typically three or four years (Paine, 1990). However, other studies used primarily unvalidated scale or length-based age estimates and may have underestimated maximum age. Maximum age reported for *E. caeruleum* based on unspecified aging methods was three years in Arkansas (Robison and Buchanan, 1984), and 3–4 years in Tennessee (Etnier and Starnes, 1993). Longevity could be greater than five years, because the largest fish collected (67 mm) was less than the 76 mm maximum reported for rainbow darters in the Ozarks region (Robison and Buchanan, 1984; Pflieger, 1997). Undetermined sampling biases could explain relatively low numbers of zero- and one-year olds. The dominant age of 2 years in collections corresponds to the age at which rainbow darters begin to defend territories and dominate spawning activities (Etnier and Starnes, 1993; Pflieger, 1997).

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