

Testing the validity of Cating's (1953) method for age determination of American shad using scales

ABSTRACT

Cating's method of using scales to age American shad (*Alosa sapidissima*) has been the standard for more than 50 years. However, the only validation of this method is for ages 4–6 in the Connecticut River. To test the method for these—and older—age classes in another river, we obtained scales from 52 known-age fish from two Pennsylvania rivers and had 13 experienced biologists estimate ages using Cating's method. Each biologist read the scale impressions twice, and these readings were then assessed in terms of precision, accuracy, and bias. Percent agreement between estimates for the same scale set (precision) ranged from 50.0 to 76.5%. Percent agreement between estimated age and known age (accuracy) was highest for ages 3–6 (33.7–48.5%), markedly lower for age-7 (12.1%), and lowest for age-8 fish (3.9%). Ages of the youngest fish were often overestimated, and those of the oldest fish were typically underestimated (bias). Therefore, Cating's method is not applicable to American shad in these Pennsylvania rivers. In fact, this scale-ageing method has never been validated across all ages for any American shad stock. Thus, we recommend against using age-based techniques to assess stocks of American shad until further age-validation studies have been completed.

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Introduction

The use of scales to age fish moved rapidly from discovery to application during the first quarter of the last century (Lee 1920; Carlander 1987). Use of scales can, however, lead to ageing error, particularly when the scale does not grow continuously in older fish or when the scale margin is resorbed in stressed fish. Researchers have also noted such problems when ageing with other hardparts, but scale-ageing methods have drawn particular criticism because of their popular use coupled with infrequent validation of the technique for many species (Summerfelt and Hall 1987). For example, Beamish and McFarlane's (1987) review found that ages derived from scales were erroneous for at least 16 species. Recent work continues to report problems with ageing fishes that had historically been aged via scale annuli (e.g., Pikitch and Demory 1988; Lowerre-Barbieri et al. 1994; Dutka-Gianelli and Murie 2001).

Nonetheless, scales continue to be used for ageing fish. This is particularly true for *Alosa* species (Clupeidae) of the North Atlantic Ocean (O'Gorman et al. 1997; Baglinière et al. 2001). In the case of American shad (*Alosa sapidissima*), scales are the only validated method to determine annual ages, although both scales and otoliths are used to age this species (e.g., Leggett and Carscadden 1978; Limburg 2001). American shad, an important fishery species in North America (Limburg et al. 2003), can be managed through age-based stock assessment

methods if a validated method of ageing this species exists. In this article, we review the earlier efforts to describe and validate a method of using scales to age American shad (Cating 1953; Judy 1961) and report on a new validation effort, which to our knowledge is only the second attempt to validate a method of ageing known-aged individuals of an *Alosa* species.

Cating (1953) described scale development and morphology of Hudson River American shad for the purpose of ageing this fishery species. Cating's method established criteria for distinguishing true winter annuli from: (1) false annuli, (2) freshwater zone marks (formed in juveniles), and (3) spawning marks (scar-like rings on scale margins considered equal in age to a true annulus but caused by erosion, absorption, or inhibition of growth when a spawning adult enters fresh water). Cating's (1953) method superseded earlier methods for ageing American shad and has been the standard for more than 50 years, largely because Judy (1961) validated the method for three age-classes of this species. In Judy's study, juveniles were marked (pelvic-fin rays clipped) and released in the Connecticut River. In subsequent years (1956–1958), he used radiography to check spawning fish for marks, and scales of recaptured fish (ages 4–6, both virgin and previously spawned) were read in a manner similar to that used by Cating (1953). Ages were 98% accurate among the 129 fish recaptured. These efforts and results are impressive, even by the standards of age-validation studies today (Campana 2001). However, it should be noted that Judy (1961) did not describe a protocol that could be considered a blind trial. That is, it is unknown if ages were determined by scale readers who were unaware of the ages in the sample.

There have been no other studies to validate adult American shad ageing methods and there are

a number of concerns that Cating's method may not apply to all populations of this widely distributed species. Discrete spawning populations of American shad exist from Maine to Florida, with populations in different biogeographic provinces (Limburg et al. 2003). American shad mature at ages 3–7, and southern populations are semelparous (Leggett and Carscadden 1978; Maki et al. 2001). Individuals in iteroparous populations of American shad may skip years between spawning, a behavior that could alter or confound interpretation of presumptive annuli on scales. Annulus- and spawning-mark formations were validated by Judy (1961) for fish up to age 6, but the species may attain 12 years of age or older in northern populations. It is unknown if spawning marks form on scales in all spawning fish, especially those with relatively short migration distances and brief spawning durations in freshwater. American shad are anadromous and make long oceanic migrations from offshore mixed-stock assemblages into natal streams to spawn (Dadswell et al. 1987; Limburg et al. 2003). These life history patterns, coupled with the fact that many stocks are depleted and under fishing moratoria (Olney and Hoenig 2001), make many validation methods (e.g., mark-recapture, marginal-increment analysis, bomb radiocarbon dating; see Campana 2001) difficult or impossible to apply. Validation from another river system would add much needed support for Cating's (1953) ageing method and Judy's (1961) mark-recapture study. Although high precision is desirable, high accuracy is required for age-based estimates of mortality, growth, maturity schedules, and production. Inaccuracy of age estimation in American shad could limit stock-assessment options and weaken management actions for this economically important species.

In anticipation of an upcoming stock assessment of American shad by the Atlantic States Marine Fisheries Commission (ASMFC), a workshop was held to validate Cating's (1953) method for ageing shad in another river system, the Lehigh River and the neighboring Schuylkill River, Pennsylvania. Known-aged fish were available from these rivers as a result of a hatchery program designed to enhance spawning runs, and the workshop was designed for experienced biologists from the U.S. Atlantic coast to age these fish as a validation experiment. We assessed each biologist's estimated ages in terms of precision, accuracy, and bias so that we could offer recommendations for estimating and using American shad ages in the future. Specifically, the findings of this experiment lead us to caution against applying age-based

assessment techniques to American shad stocks that lack age validation across all age classes.

Methods

American shad larvae were cultured using methods similar to those reported by Howey (1985): ripe adults were collected in the Delaware River at river kilometer (rkm) 351 (as measured from the center of a line between Cape May and Cape Henlopen) and strip-spawned, and the resulting eggs and larvae were cultured at the Pennsylvania Fish and Boat Commission's Van Dyke Hatchery. Larvae were fed optimal diets (Wiggins et al. 1986), marked on multiple days with tetracycline, and stocked during May or June at 7–21 d of age in the Lehigh or Schuylkill rivers (Figure 1) beginning in 1985. Hatchery cohorts from 1995 to 2000 were given unique mark patterns to identify year stocked (Table 1). Releases occurred at Northampton (Lehigh River rkm 38.6) or between Gibraltar and Hamburg (Schuylkill River rkm 108–158). The fish

Figure 1. A map of the Delaware River Basin depicting the sites for collecting adult American shad and releasing cultured larvae.



Table 1. Tetracycline marking of American shad larvae released in the Lehigh and Schuylkill rivers, 1995–2000. Marking occurred on a single (day 5) or multiple days (3–18) post-hatching to create a bar-code-like pattern to identify each fish's year class. Details of marking methods are reported in Hendricks et al. (1991).

Stocking site	Year	Age at marking (days)	Number stocked	Number of study specimens by year of recapture				
				1999	2000	2001	2002	2003
Lehigh River	1995	5	1,044,000	5	9	9	10	3
	1996	3, 6, 9	993,000		2		1	
	1997	5, 9, 13	1,247,000		1	1	1	
	1998	3, 9, 12	948,000					
	1999	9,12,15	501,000					2
	2000	3,6,9,12,18	447,390					
Schuylkill River	1999	3, 9, 12	410,000					
	2000	3,6,9,12	536,000					8

used in this experiment were from fish stocked in both rivers.

Adult American shad were collected using boat-mounted electrofishers or gill nets in April–June of 1999–2003 and scales were removed for age analysis. Adult sampling sites included the Delaware River at Raubsville (rkm 286) and Smithfield Beach (rkm 351), the Lehigh River below Chain Dam (rkm 4.8), and the Schuylkill River below Fairmount Dam (rkm 14, Figure 1). Scales were collected from the area below the dorsal fin and above the lateral line and stored dry in labeled envelopes. Three to five scales from each fish were cleaned and impressions were created by pressing the scales sculptured side down on an acetate slide (1.27 mm thick) using pressure (5000 psi) and heat (100° C) for 5 min.

The otoliths were also saved to check for recaptures and assign year class. Sagittal otoliths were extracted from previously frozen fish heads, mounted on a microscope slide, and ground on both sides to produce a thin sagittal section (Hendricks et al. 1991). Otoliths were examined by a single reader (MLH) to identify the presence and pattern of a tetracycline mark, using an epifluorescent microscope with a

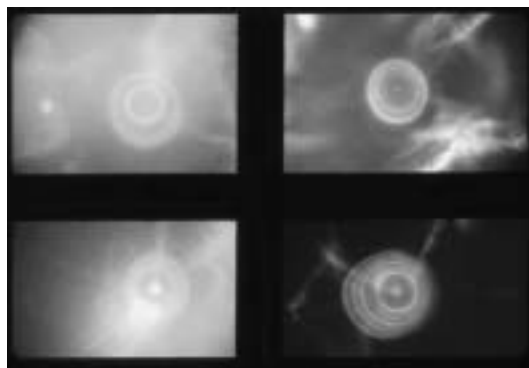
100W mercury vapor lamp and an fluorescein isothiocyanate fluorcluster under UV light (Figure 2). Therefore, estimated ages referred to in this article are based on scales only; otoliths were used only to detect the cohort-specific tetracycline marks and thereby assign a known age as the difference between the year the fish had been released and the year it was recaptured (Table 1).

The validity of this experiment relies on the assumption that the age of each American shad is known. There are three mechanisms by which unknown-age fish could be mistaken for known-age fish: straying, misreading of marks, and data errors. We conclude, however, that none of these issues were a significant problem for the following reasons. In terms of straying, specifically straying to systems with similar marks, the unique tetracycline marks that characterized known-age fish in the Delaware River system were duplicated in fish stocked in the Susquehanna River Basin in other years. Thus, strays from the Susquehanna River would not be distinguishable from known-age fish from the Delaware River system. Straying of tetracycline-marked fish from the Susquehanna River to the Delaware River has been documented but is extremely rare. Between 1990 and 1999, some 30,824,800 American shad larvae were stocked in the Susquehanna River bearing marks that were never used in the Delaware, Lehigh, or Schuylkill rivers. Only 2 (0.00001%) of these fish were recaptured as strays in the Delaware River from 1995 to 2003. During the same period, 58,826,700 larvae were stocked in the Susquehanna River bearing marks that were also used for fish stocked in the Delaware River basin. Assuming a similar recapture rate, we would expect 3.8 Susquehanna strays bearing marks that were used in both the Susquehanna and Delaware systems in our sample of 3,297 specimens. Thus, the probability that one of our test specimens is a stray from the Susquehanna is extremely low ($0.001 = 3.8/3,297$). The uniqueness of the tetracycline marks used in this study derives from the number of tetracycline marks and their spacing. Anomalies in otolith growth or poor grinds can make the marks difficult to read, but these cases are rare. Most of the marks are easily read. Errors in data recording and specimen labeling or mounting are rare but possible in any biological field operation. Overall, it was extremely unlikely that the results of our experiment were affected by such potential sources of error.

The age-validation experiment was designed so that the biologists evaluating the scale impressions did not know the range of age classes or the number of fish per age class. A sample of scale impressions was randomly chosen to select for nine age-3 fish, ten fish per each age class 4–7, and three age-8 fish ($n=52$). Because Cating reported that his methods could be applied to all scales, the only fish rejected during our selection process were those dominated by regenerated scales. The final sample of scale impressions was shuffled to randomly mix the order of age classes and then labeled in consecutive order (i.e., 1 to n).

Figure 2. A sample of tetracycline marking sequences in American shad otoliths. Marks were applied by 4h immersion in 256 mg/L oxytetracycline at age (days) listed.

- upper left—3, 9, 12
- upper right—5, 9
- lower left—3, 6, 9
- lower right—3, 6, 9, 12, 15



American shad, an important fishery species in North America, can be managed through age-based stock assessment methods if a validated method of ageing this species exists.

Thirteen biologists from Florida to Maine were assembled to read this sample of scale impressions. These biologists' years of experience in ageing shad ranged from 2 to 25 years (median 7 years). Each biologist evaluated 4–6 sets of scale impressions at each of 11 different microfiche stations (and 2 biologists rested during each rotation). Each biologist read the entire sample (i.e., all 52 sets of scale impressions), once during the morning, and a second, independent evaluation was completed in the afternoon. Statistical independence was ensured during the afternoon session by changing the consecutive identifying code on all 52 sets of scale impressions and by moving some sets to different stations.

Individual performance by each biologist was initially screened—in relation to precision, accuracy, and bias—using age bias plots (Campana et al. 1995). Precision (i.e., repeatability between the first and second age estimate for the same fish) was also measured as the percent of replicate (paired), estimated ages that agreed exactly (i.e., percent precision), and as the index of coefficient of variation (ICV; Chang 1982):

$$100 \times \frac{1}{N} \sum_{j=1}^N \sqrt{\frac{\sum_{i=1}^R (Y_{ij} - \bar{Y}_j)^2}{R-1}} \frac{1}{\bar{Y}_j}$$

where N is the number of fish aged, R is the number of replicated age estimates per fish, Y_{ij} is the i th age determination of the j th fish, and \bar{Y}_j is the average age for the j th fish. High precision is indicated by high values of percent precision and low values of ICV.

Accuracy was measured as the percent of estimated ages that agreed exactly with the known age (i.e., percent accuracy). This measure of accuracy included all ages without reconciling differences between paired age estimates to determine a "single" age per fish. Bias was evaluated by using regression analysis of all estimated ages versus known age (Sokal and Rohlf 1981) and tests of symmetry from age-frequency tables (Hoenig et al. 1995). Regression analysis of estimated ages as a function of known age was calculated by model I least squares regression ($H_0: \text{slope} = 1$). Bowker's (1948) test of

symmetry tested the hypothesis that the observed error in ages was randomly distributed along the table diagonal for an $r \times c$ matrix, where columns are known ages and rows are estimated ages. Significance of each biologist's performance was evaluated at $p = 0.0038$ to adjust for a total alpha value of 0.05 across all 13 biologists.

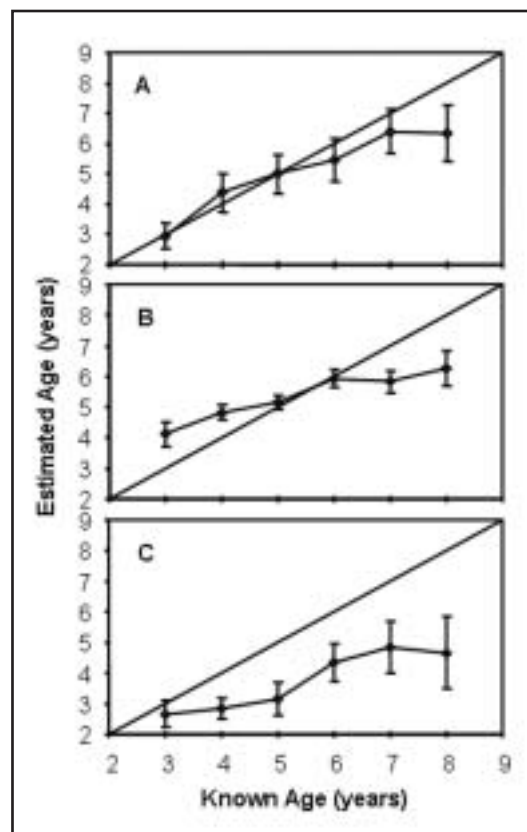
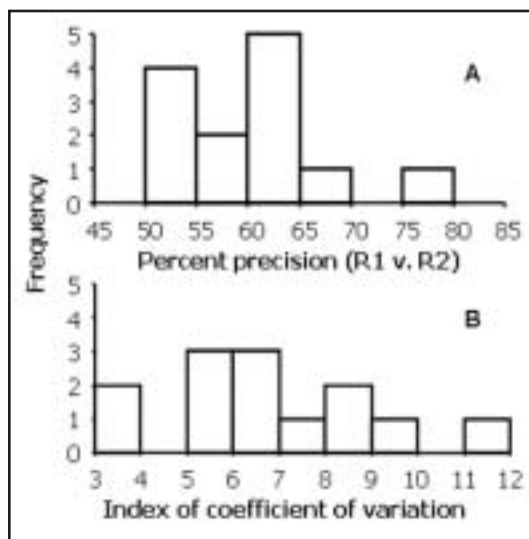
Results and Discussion

Percent precision ranged from 50.0 to 76.5% (mean = 59.7) and the ICV varied between 3.79 and 11.08 (mean = 6.9) for each biologist (Figure 3). Campana (2001) reports that the modal ICV for 117 published studies is 5, which he suggests as a suitable reference point. The modal ICV for this experiment was 6.0. Thus, the biologists in this experiment were ageing this sample of shad scales with good precision.

Only 31.8% of all estimated ages were accurate. Percent accuracy was highest for age-3 fish (48.5%), ranged from 33.7 to 40.6% for age-4 to age-6, dropped noticeably for age-7 fish (12.1%), and was lowest for age-8 fish (3.9%). The abilities of the readers to estimate ages accurately varied substantially (Figure 4).

Figure 4. Age bias plots for three biologists representing the different types of performance at estimating age of American shad from scales: (A) one of the best performances, where ages of only age-8 fish are severely underestimated, (B) a representative of the "typical" performance type observed in this study (i.e., overestimated ages for younger fish and underestimated ages for older fish), (C) one of the poorer performances, where nearly all ages were severely underestimated. The data are for mean estimated age ($\pm 95\%$ confidence limits) for a sample of known-aged fish. The diagonal line defines unity (i.e., correct estimate of age).

Figure 3. Measures of precision performance by 13 biologists estimating ages from American shad scales: (A) percent precision, (B) index of coefficient of variation. "R1 vs. R2" = first age estimate versus second age estimate for the same set of scale impressions.



Although several biologists were able to accurately age >50% of the younger ages (ages 3–6), the modal accuracy was lower, and a few biologists correctly aged less than 10% of these younger known-aged fish (Figure 5a). The most accurate performance for ages 7 and 8 was 20–30%, and most of the biologists could not correctly estimate the ages of older fish more than 10% of the time.

The inaccuracies in estimated ages were biased (Table 2). Although about half (48.5%) of the biologists were correctly ageing the age-3 fish, nearly all the inaccurate estimates for age-3 fish were overestimates (i.e., 45.5% of the age-3 ages were estimated as either age-4 or age-5). Among older ages, the opposite bias occurred. For age-8 fish, there were no overestimates, and 96.1% of the estimated ages were underestimates. For age-7 fish, 4.3% of the reads were overestimates, and 83.6% were underestimates. These patterns were confirmed with a regression analysis of estimated age versus known age, which showed across all readers that the slopes were significantly less than one (but significantly greater than zero), ranging from 0.25 to 0.73 (Figure 5b). The tests of symmetry confirmed this conclusion of poor associations between the estimated ages and the known ages made by each biologist: 11 of the 13 comparisons were significantly asymmetrical (Figure 5c).

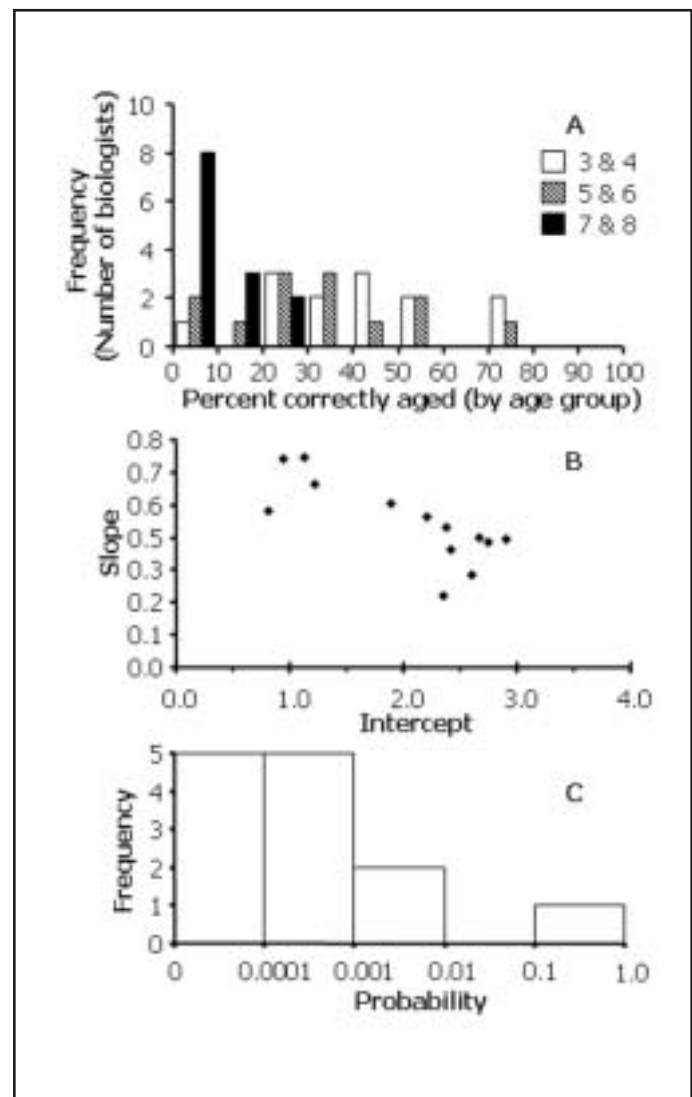
In summary, Cating's (1953) method was not suitable for ageing these scale impressions of American shad from two Pennsylvania rivers. This experiment found much lower accuracy for ages 4–6 than that reported by Judy (1961) for Connecticut River shad. Moreover, accuracy was even lower in older fish (age-7 and -8) and an asymmetrical bias was evident for both the youngest and oldest age-classes. The shortcomings identified here are not unusual for scale ages (see introduction), and a general need for more research and validation of ageing methods is still, regrettably, the norm for many fishes (Campana 2001).

What is wrong? The likely explanation for these results resides with process error and/or observation error. Process error occurs when the structure selected lacks all the pertinent landmarks used for ageing, such as when some annuli are missing or cannot be distinguished (e.g., Lowerre-Barbieri et al. 1994; Long and Fisher 2001). Observational error is caused by the incorrect interpretation of a suitable ageing structure (e.g., Bailey et al. 1977; Mann and Steinmetz 1985). Several biologists noted that the scales used in this experiment had

Figure 5. Measures of accuracy and bias for 13 biologists estimating ages from American shad scales. (A) Percent accuracy (to exact year but depicted for three different age groups [ages 3 and 4 fish pooled, etc.] to show effects of fish age). (B) The association between regression slopes and intercepts for the equation: Age[estimate] = a + b × Age[known]. (C) Frequency of probabilities for rejecting a symmetrical distribution of paired, estimated ages versus known age; 11 cases were rejected ($p < 0.0038$).

Table 2. Age estimates for known-aged fish ($n = 52$), pooled for all replicate reads ($n = 2$) and all biologists ($n = 13$). Total number of estimated ages should be 1,352 (i.e., $52 \times 2 \times 13$), but some biologists chose not to record an age for some fish so the final sample size is slightly smaller ($n = 1,322$).


Known age (year)	Estimated age (year)						
	2	3	4	5	6	7	8
Frequency	14	113	100	6	0	0	0
Row Pct.	6.01	48.5	42.9	2.58	0.00	0.00	0.00
3	5	51	99	80	8	1	0
4	2.05	20.9	40.5	32.7	3.28	0.41	0.00
5	3	37	98	86	29	2	0
6	1.18	14.5	38.4	33.7	11.3	0.78	0.00
7	0	10	41	106	88	9	3
8	0.00	3.89	15.9	41.2	34.2	3.50	1.17
0	0	6	37	76	95	31	11
1	0.00	2.34	14.4	29.6	37.1	12.1	4.30
2	0	1	9	21	32	11	3
3	0.00	1.30	11.6	27.2	41.5	14.2	3.90



highly eroded or resorbed margins, and it was largely agreed that this was due to the majority of these fish being collected on the spawning grounds, well upstream in the Lehigh or Delaware rivers (in contrast, many other monitoring programs sample on shorter rivers or collect their fish in the lower reaches of the river). Spawning marks were postulated by Cating (1953) to be caused by erosion processes while the fish are in freshwater. A serious problem would result if this erosion obscured previously laid annuli or spawning marks. Even if this erosion does not completely obscure previously laid annuli (i.e., process error), it may reduce the resolution of closely spaced annuli along the margin and thereby contribute to observation error. The general laboratory conditions in our validation trial, although ensuring a strong statistical design, did not create ideal working conditions for the biologists and may also have contributed to observation error. Some microfiche machines were not optimal, some evaluations may have been hurried, and some biologists were not comfortable with plastic impressions, so performance may have suffered as a result. Nonetheless, these conditions do not appear to account for the strong bias evident in the results. At the end of this rigorous, blind experiment, several images of scales were projected on a large screen and the assembled biologists still could not identify the annuli to correctly age the oldest individuals. Contrasting examples of scales with high versus low precision and accuracy in this experiment are depicted in Figure 6.

We conclude that Cating's method may not be universally applicable to American shad from all rivers and at all ages. Currently no successful age-validation study exists for American shad across a representative age range. Based on these findings, we strongly recommend:

- (1) Scientists should use caution in applying age-based assessment techniques for American shad age data. Overestimating the age of young fish coupled with underestimating the age of older fish could artificially increase mortality estimates (Eklund et al. 2000), increase growth coefficients (as reported in ASMFC 1998), skew maturation schedules (Maki et al. 2001), reduce estimates of production (Boreman and Friedland 2003), and confound models of population dynamics (Gibson and Myers 2003). In other studies, scales are used even when errors are evident because scales are easy to collect, and with proper handling, the fish are not injured or killed. Ageing error can be corrected in two ways. If the distributions of inaccurate reads are normally distributed around mean values, then an ageing error matrix can be used to statistically remove this error (Richards et al. 1992; Heifetz et al. 1999). Or if an ageing method fails after fish reach a certain age, then older age classes can be pooled together before applying assessment models (Welch et al. 1993; Secor et al. 1995; VanderKooy and Guindon-Tisdell 2003). The low accuracy and severe biases encountered in this experiment do not, however, suggest that these approaches will be helpful.
- (2) A better understanding of the processes that form transverse grooves and spawning marks in American shad scales is required. The main criterion identified by Cating (1953:195) was that the first three annuli must fall within three non-overlapping ranges of transverse-groove counts. Transverse grooves are "distinct grooves in the surface of the anterior, sculptured portion, crossing it laterally in the same general contour as the striae, but spaced farther apart" (Cating 1953:190). Cating postulated that subsequent annuli are proportionally spaced until spawning begins. Thereafter, the anterior and lateral scale margins become eroded



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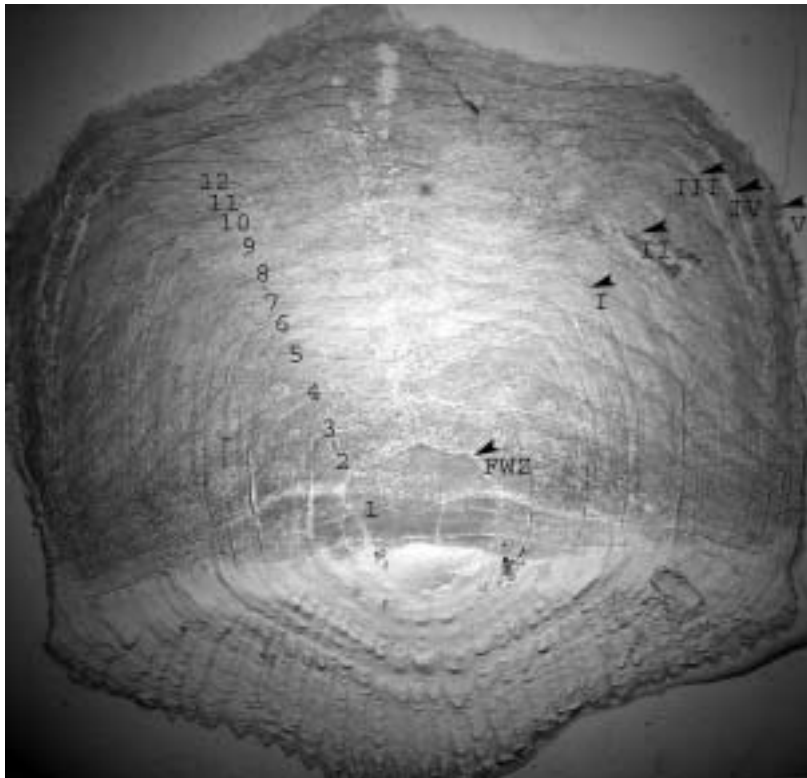
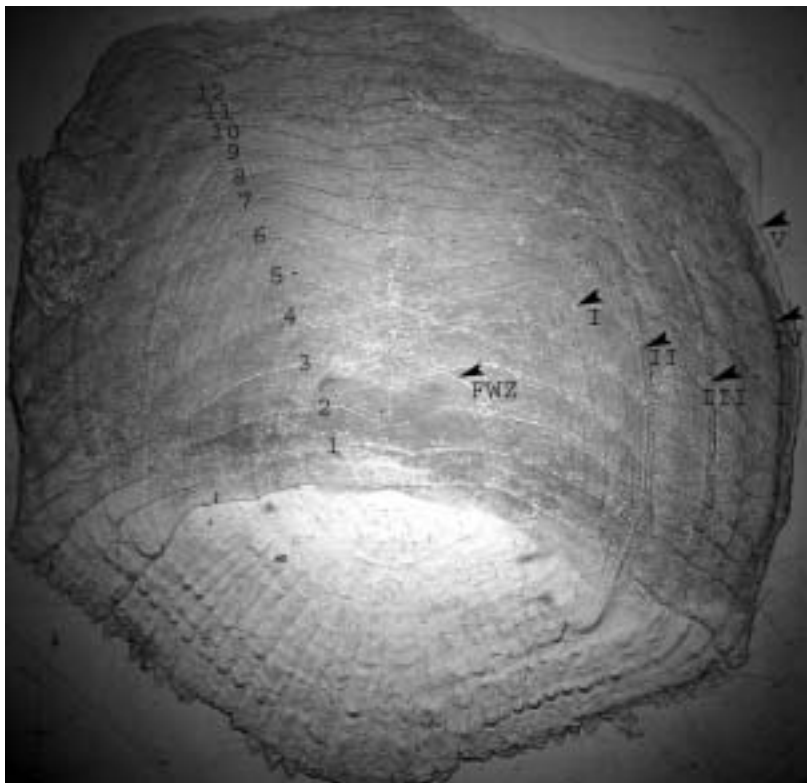
Figure 6. Contrasting examples of American shad scales.

TOP—A 5-year-old (known age) aged with high accuracy and precision (average estimated age = 4.8 and ICV = 5.9 among the 13 biologists). BOTTOM—A 7-year old (known age) aged with low accuracy and precision (average estimated age = 5.1, ICV = 9.0).

Landmarks are indicated in each image as:

FWZ = Freshwater zone; 1–12 = transverse grooves; I–V = presumptive annuli as determined by Cating's method.

Images and interpretation provided by B. Watkins.



during the period of spawning in freshwater, changing the proportional spacing between spawning marks. It is possible that natural variability in the formation of transverse grooves, annuli, and spawning marks confounds the application of Cating's method to other river systems.

- (3) Biologists reluctant to abandon the scale ageing method should work to build reference sets of scales from known-aged American shad for additional, stock-specific training and validation sessions (Buckmeier 2002). To obtain suitable reference sets, hatchery managers charged with American shad restoration projects should release larvae or juveniles with year-specific marks annually. This is a critical need that should receive priority over other hatchery-based restoration goals. Other corroborative approaches for examining the assumptions of annulus formation could also be useful in the absence of having known-aged fish (Campana et al. 1995; Lai et al. 1996; Campana 2001).
- (4) Otoliths should be evaluated as an alternative method of ageing American shad. If known-age specimens are unavailable, otolith microchemistry, particularly strontium chronology, may offer opportunities for age validation (Secor and Rooker 2000).

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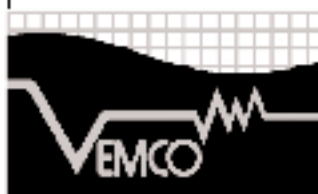
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