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NOTES

A Comparison of Calcified Structures for Aging Bluefish in the Chesapeake Bay Region

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Abstract.—We compared whole and sectioned otoliths, scales, dorsal spine sections, opercular bones, and vertebrae for aging bluefish *Pomatomus saltatrix* from the Chesapeake Bay region. Opercular bones and vertebrae were unusable; they were spongy and pitted, and contained no visible marks. The remaining structures exhibited concentric rings that were interpreted as annual marks; however, structures differed greatly in mark clarity. Over the age range 1–14 years, sectioned otoliths were the best structure, consistently showing the clearest marks, highest confidence scores, and highest within- and between-reader agreement. Whole otoliths were the second best structure, providing the second highest overall within-reader agreement and 95% agreement with sectioned otoliths to age 4. Scales were inferior to sectioned and whole otoliths, especially after age 4, when within-reader agreement was only 33% and agreement with sectioned otoliths was only 26%. Dorsal spine sections were undesirable for aging bluefish, providing the lowest reader agreement and exhibiting unclear, inconsistent marks.

The bluefish *Pomatomus saltatrix* is one of the most economically important fishes on the Atlantic coast (Wilk 1977; MAFMC 1990). As such, proper aging is essential for their management. Although many studies have reported age determination in bluefish, few have attempted to compare calcified structures for aging. Before 1980, only scales were used for aging bluefish (Hamer 1959; Backus 1962; Lassiter 1962; Richards 1976; Wilk 1977). Problems have been reported with scale use, however, including high incidences of regeneration, false annuli, and different age readings between scales from the same fish (Backus 1962; Lassiter 1962; Richards 1976). In addition, no studies have reported precision in repeated readings for scales or presented data to validate them.

More recent studies have examined other structures for aging bluefish. Barger (1990) compared calcified structures in bluefish from the Gulf of Mexico and found that whole otoliths yielded much higher between-reader agreement (92%) than did scales (67%). However, Barger did not report between-structure agreement or the age range of fish used in his comparison, making it difficult to evaluate whether these structures were useful at both young and older ages. Chiarella and Conover (1990) reported 93% agreement between scales and sectioned otoliths in 29 bluefish age 6 and younger caught off Long Island, New York. Neither Barger (1990) nor Chiarella and Conover (1990) evaluated otoliths at older ages, however, even though scales have often been shown to underage compared with otoliths at older ages (Beamish and McFarlane 1983; Lowerre-Barbieri et al. 1994; Ihde 2000; Sipe and Chittenden 2001).

The length-frequency method MULTIFAN has also been used to age bluefish (Terceiro and Ross 1993). However, this method is problematic for two reasons: (1) scale ages are used as initial input, and (2) it requires clearly separated modal groups to separate the length frequency into component age-groups, a requirement that is difficult to satisfy at older ages.

Further evaluation of bluefish calcified structures is needed to determine an optimal aging structure, especially given the economic importance of this species and reported difficulties in age determination with scales. The objective of our study was, therefore, to evaluate and compare whole and sectioned otoliths, scales, dorsal spines, opercular bones, and vertebrae for aging both small and large bluefish. Structures were evaluated using the following criteria: presence and clarity of presumed annual marks, precision in repeated age readings, agreement between different structures of the same fish, and increases in structure size and age with fish growth.

Methods

Bluefish were collected from commercial fisheries and recreational tournament sources in the Chesapeake Bay region in May and June 1999

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(N = 63) and September through November 1999 (N = 43). Additional unusually large fish were collected in February 2000 near Cape Hatteras, North Carolina (N = 8). To include as many age-groups as possible in the final study sample, the 1999 collection was stratified into three length-based categories: 201–400 mm, 401–600 mm, and >600 mm fork length (FL). A random sample of 20 fish was chosen from each category. These 60 fish were supplemented with the five largest fish of those collected in February 2000, bringing the total final study sample to 65 fish, ranging from 226 to 864 mm FL and 1–14 years (sectioned otolith age).

For each fish, we measured fork length and weight and removed scales, both sagittal otoliths, the spinous dorsal fin, opercular bones, and the entire vertebral column. We took scales from near the tip of the left pectoral fin below the lateral line (Richards 1976). Calcified structures were stored and prepared for aging as described in Sipe (2001).

Two readers examined and aged each structure after doing practice readings of each structure. Structures were read in haphazard order without knowledge of fish size or collection date. Different structures from the same fish were read independently (i.e., without knowledge of or associations with other structures from the same fish). After practice readings, structures from each fish were read twice by reader one and once by reader two. At least 1 week separated the first and second readings of the same structure. Ages were assigned based on presumed annual mark counts (hereafter referred to as marks). Reader comments on structures were used to determine the cause of disagreements within and between readers.

Dorsal spine sections and whole and sectioned otoliths were examined under a compound microscope using both reflected and transmitted light at 120–1,000× magnification, depending on the size of the structure. Marks were identified as opaque bands, which appeared dark in transmitted light and white in reflected light. On whole otoliths, marks were counted from the focus to both the anterior and posterior margins of the otolith. On sectioned otoliths, marks were counted along the dorsal side of the sulcal groove. Scale impressions were read with a Bell-Howell R753 microfiche reader at 20× and 32× magnification. Marks on scales were identified using standard criteria as described in Bagenal and Tesch (1978).

Mark clarity was evaluated with confidence scores and reading times. Confidence scores were assigned by the reader to every structure using a scale of 1 (low) to 5 (high). Between-structure differences in confidence scores were tested at $\alpha = 0.05$ with the normal approximation to the Mann–Whitney test for ordinal data (Zar 1996).

Precision in age determinations for each structure was evaluated with simple percent agreement in repeated readings within and between readers. Agreement ±1 year was also calculated to indicate how widely age estimates diverged. Within-reader agreement compared the first and second readings by reader one. Structure readings that disagreed in the first two readings by reader one were reread independently by the same reader to reach a consensus age. Between-reader agreement compared the consensus age of reader one with the age determined by reader two.

Agreement in mark counts between different structures of an individual fish was evaluated by simple percent agreement between structures and simple linear regression. Consensus ages for one structure were regressed on consensus ages for another structure, and the slope of the regression line was tested to see if it differed significantly from 1. A slope of 1 implies that $y = x$ and that the two structures provide the same age. The x-variable was the structure judged superior based on confidence scores and within- and between-reader agreement.

Calcified structures were measured with a calibrated digital imaging system and SPOT RT software version 3.0 (Diagnostic Instruments, Inc. 1999). Dorsal spines were measured for sectional surface area (DSSA) and vascular core area. Scale radial length (ScRL) was measured from the center of the focus along the ventral axis where the anterior marks curve down to the lateral field. Whole otolith total length (WOTL) was measured from the posterior to anterior margin through the center of the focus, and sectioned otolith radial length (SORL) was measured along the dorsal arm of the sulcal groove from the center of the focus to the otolith edge. Otoliths that were fractured along the measuring path were not measured.

The assumptions that structure growth and the number of marks on a structure are directly related to fish growth were evaluated with linear regression (Zar 1996). To determine if these relationships were significant and increasing, structure size (DSSA, ScRL, WOTL, SORL) and the number of marks on a structure were regressed on fork length.

Results

Comparative Appearance of Calcified Structures

Initial evaluations indicated that opercular bones and vertebral sections could not be used for

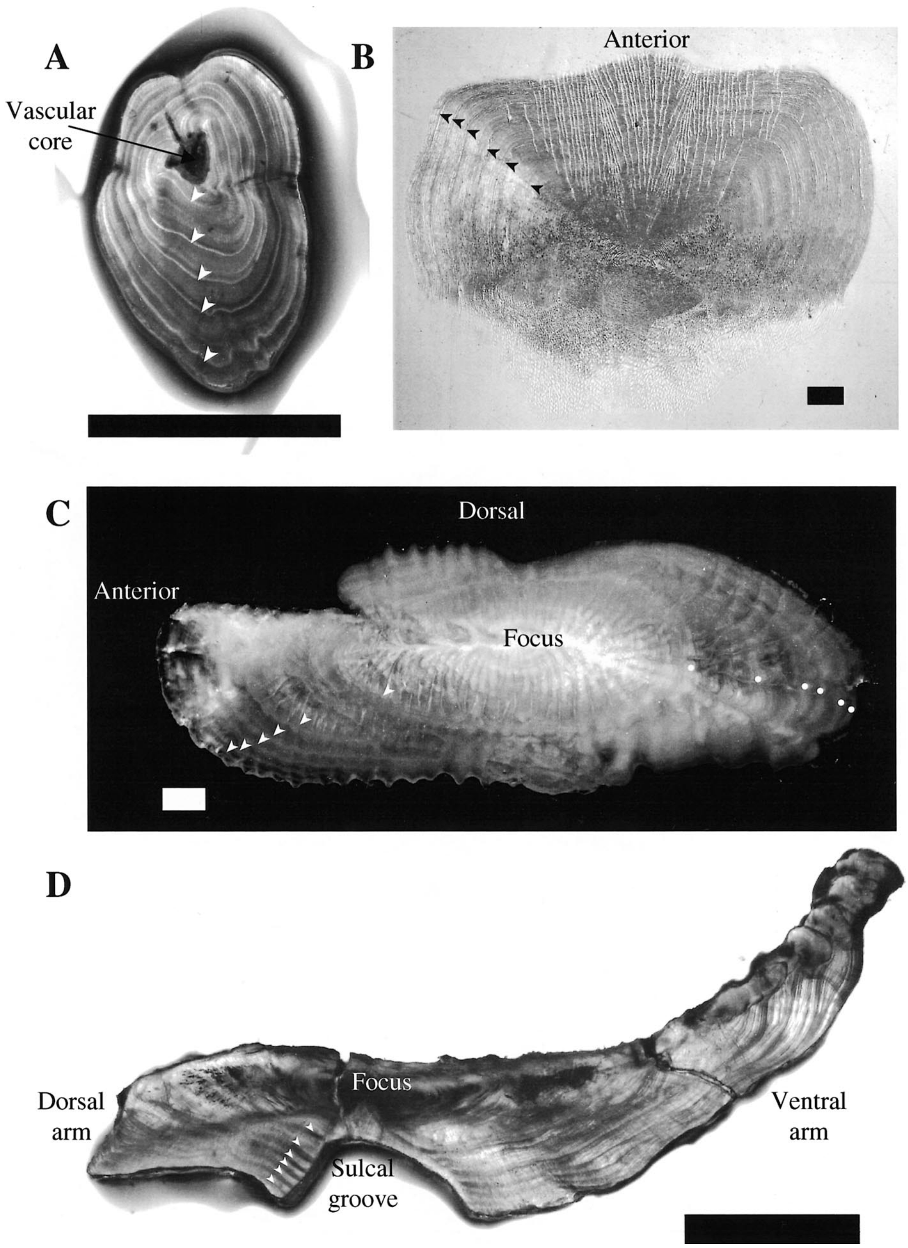


FIGURE 1.—Presumed annual marks (arrowheads) on calcified structures taken from a 6-year-old female bluefish (as aged by sectioned otolith; 724 mm fork length, collected 22 November 1999): **(A)** dorsal spine section viewed with transmitted light; **(B)** scale impression viewed with transmitted light; **(C)** whole otolith viewed with reflected light on a black background (arrowheads indicate marks in the anterior field, dots indicate marks in the posterior field); and **(D)** transverse otolith section viewed with transmitted light. Bars = 1 mm.

aging because they were yellowed and spongy and contained no visible marks. As a result, these structures were eliminated from further comparisons. Dorsal spines, scales, and whole and sectioned otoliths all showed concentric marks that could be interpreted as annual marks (Figure 1). However, these structures differed greatly in mark clarity, with age generally the biggest factor and younger fish being the easiest to interpret.

Marks on dorsal spine sections (Figure 1A) were fairly clear in some fish but were more often poorly defined and inconsistent. The first mark was particularly difficult to identify on young fish and was a large source of disagreement between readers and between structures. Common problems encountered with dorsal spine sections from large fish included a large vascular core, light and narrow edge marks, and poor separation of marks.

Marks on scales (Figure 1B) were typically clear and easy to read in younger fish, but age determination from scales of older fish was difficult and more subjective. False marks, indicated by crossing over in only one lateral field, were a common problem with scales, particularly in younger fish. Age interpretation in older fish was hampered because of crowding of marks at the scale edge and a thick and pitted scale center that obscured early marks. In addition, many fish had regenerated, asymmetrical, or otherwise damaged scales.

Marks on whole otoliths (Figure 1C) were fairly clear in younger fish but became increasingly difficult to identify in older fish. Unlike dorsal spines, the first mark was generally easy to identify on whole otoliths, especially in younger fish. Whole otoliths were often opaque in older fish, however, making age determination for these fish difficult and highly subjective. Older fish also tended to exhibit crowded or faint marks at the structure's edge.

Marks on sectioned otoliths (Figure 1D) were typically the clearest, most consistent, and easiest to interpret, particularly in younger fish. Disagreements in sectioned otolith ages were more common at older ages, usually reflecting a lightening of marks towards the structure's edge. This did not occur in all older fish, but it was a common source of disagreement when it did occur.

Clarity of Marks

The clarity of marks, indicated by confidence scores and reading times, varied among structures. Confidence scores were generally low and decreased with age. Sectioned otoliths had the highest overall confidence scores, averaging 4.1 (Table 1), which was significantly higher than those of all other structures ($P < 0.001$). Overall confidence scores for whole otoliths, scales, and dorsal spines ranged from 2.8 to 3.0 and were not significantly different from one another ($P > 0.381$). For all structures, confidence scores were higher at younger ages (age 4 or less) than at older ages (age 5 and older). Sectioned otoliths had the highest scores by age, averaging 4.3 at younger ages and 3.7 at older ages, significantly higher than all other structures in both age-groups ($P \leq 0.001$). Reading times were fairly high for all structures, although sectioned and whole otoliths had the shortest average reading time (1.7 min/fish; Table 1). Scales and dorsal spines required slightly more time to read (1.8 and 2.0 min/fish, respectively).

Agreement in Age Determinations for the Same Structure

Within-reader agreement (precision) in repeated age determinations varied among calcified structures. Complete within-reader agreement was highest (86%) for sectioned otoliths, followed by whole otoliths (74%), scales (67%), and dorsal spines (63%). Most within-reader disagreements were within 1 year of each other; within-reader agreement $\pm$ 1 year was 100% for sectioned otoliths and over 90% for whole otoliths, scales, and dorsal spines.

Within-reader precision in repeated age determinations also varied with age. For all structures, within-reader complete agreement was fairly high in younger fish ($\leq$4 years old). Sectioned otoliths had the highest agreement in younger fish (95%), followed by whole otoliths (88%), scales (83%), and dorsal spines (71%). For older fish ($>$4 years), within-reader agreement decreased greatly. Sectioned otolith agreement was the highest for older fish (72%), compared with only 52% for whole otoliths, 50% for dorsal spines, and only 33% for scales.

Between-reader agreement varied among calcified structures and was generally lower than within-reader agreement. Agreement between readers was highest (83%) for sectioned otoliths. Agreement was much lower for whole otoliths (62%) and scales (62%), and it was lowest for dorsal spines (38%), reflecting the overall poor mark clarity of dorsal spines compared with other structures. Between-reader agreement $\pm$1 year was 97% for sectioned otoliths and scales, but it was only 85% for whole otoliths and 77% for dorsal spines.

Comparisons of Calcified Structures from the Same Fish

Different structures from the same bluefish often did not give the same age estimates. For all structure comparisons, the null hypothesis that the slope of the regression line equals 1 was rejected ($P < 0.001$), indicating that scales, whole otoliths, and dorsal spines gave different ages than sectioned otoliths (Figure 2), which was the structure with the highest confidence and within- and between-

TABLE 1.—Average ± SE age-reading time and confidence score for calcified structures in bluefish. Confidence scores, which range from 1 (low) to 5 (high), were assigned by the reader.

Structure	Reading time (min)	Confidence score		
		Overall	≤Age 4	>Age 4
Dorsal spines	2.0 ± 0.3	2.8 ± 0.2	3.0 ± 0.2	2.6 ± 0.3
Scales	1.8 ± 0.2	3.0 ± 0.2	3.6 ± 0.2	1.9 ± 0.2
Whole otoliths	1.7 ± 0.2	3.0 ± 0.2	3.6 ± 0.2	2.2 ± 0.2
Sectioned otoliths	1.7 ± 0.2	4.1 ± 0.1	4.3 ± 0.1	3.7 ± 0.2

reader agreement. Percent agreement between structures varied with age, with all structures showing much higher agreement with sectioned otoliths at younger ages (68–95%) than at ages greater than 4 years (26–32%).

Whole and sectioned otoliths showed the highest agreement (71%) in the number of marks. Agreement was high for fish under age 4 (95%), but it decreased to only 32% in fish over age 4 (Figure 2A). When counts disagreed in older fish, ages were usually lower for whole than for sectioned otoliths due to crowding of edge marks and increased otolith opacity.

Scales and sectioned otoliths yielded the second-highest agreement (65%) in the number of marks. Agreement was high for younger fish (88%) but decreased to only 26% in fish older than age 4 (Figure 2B). In disagreements for older fish, scale ages were lower than sectioned otolith ages, largely due to mark crowding at the scale edge and scale thickening.

Dorsal spines and sectioned otoliths showed the lowest agreement (52%) in the number of marks. Agreement was only 68% in fish under age 4 (Figure 1C), and in all disagreements among younger fish, dorsal spine ages were lower than sectioned otolith ages; this was due to difficulties identifying the first mark on dorsal spines. Agreement between dorsal spines and sectioned otoliths was only 28% in fish over age 4. Again, dorsal spines aged lower than sectioned otoliths in most disagreements due to erosion of early marks and crowding of later marks in larger fish.

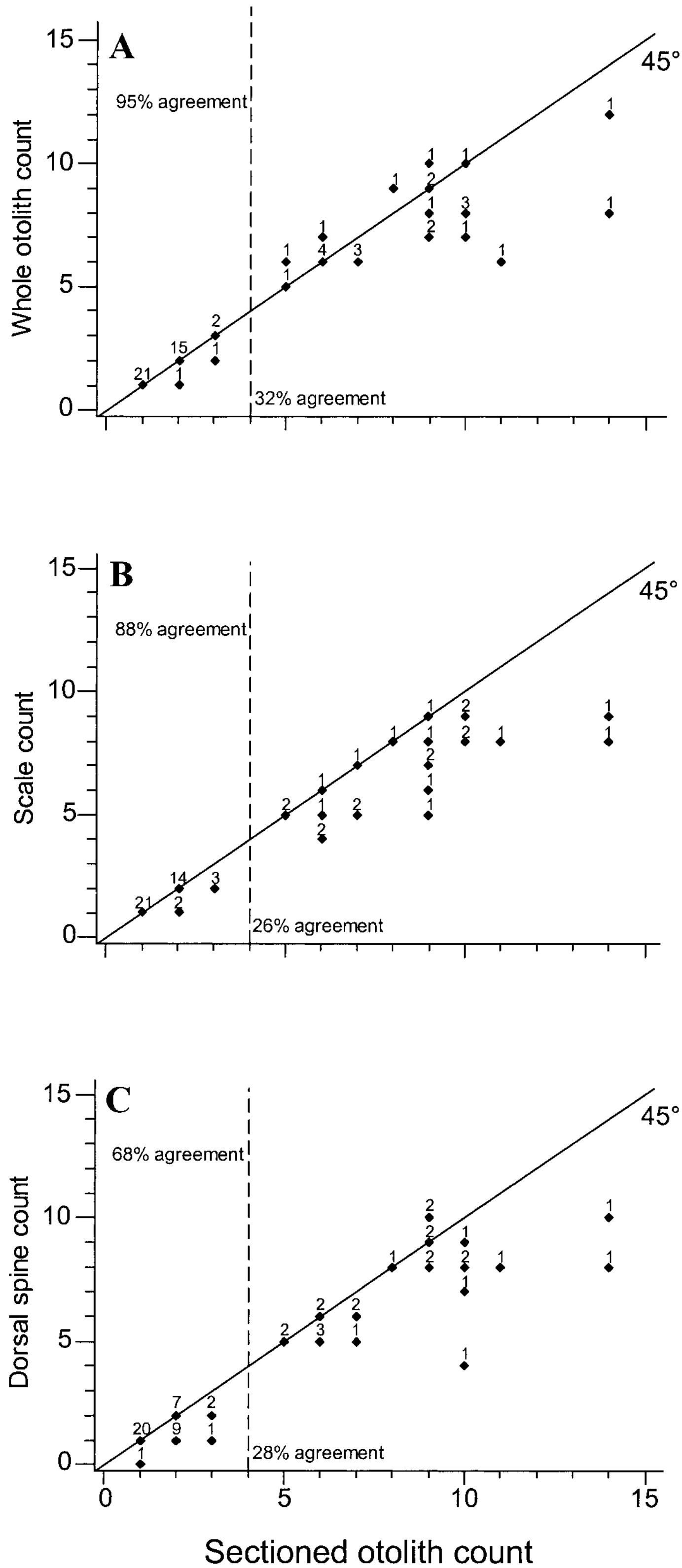
Increase in Structure Size and Number of Marks with Fish Growth

All calcified structures increased in size and in the number of marks as bluefish body length increased. All regressions of mark counts on fish size were significant ($P < 0.001$), and all slopes were positive, with coefficient of determination ($100 \cdot r^2$) values of 79–82%. Likewise, all regressions of structure size on fish fork length were significant ($P < 0.001$), and all slopes were positive, with coefficient of determination values of 86–93%. Regression of the core area of dorsal spine sections on fork length, however, also gave significant results ($P < 0.001$), suggesting that growth of the vascular core might obscure or erode early marks. Comparisons of core area with total spine area showed the core area in fish over 800 mm FL to be as large as the total spine area in fish under 500 mm FL.

Discussion

Our findings indicate that sectioned otoliths are the best structure for aging bluefish over the age range of 1–14 years. Sectioned otoliths were superior in all criteria we used to evaluate calcified structures, despite some difficulties at older ages. They were consistently clearer and easier to interpret than whole otoliths, scales, and dorsal spines, and they had the highest within- and between-reader agreement and the highest confidence scores at all ages. These findings are new because no previous studies have examined sectioned otoliths of bluefish in as great detail or over such a large age range. They agree with other species investigations that have found sectioned otoliths to be the best aging structure (e.g., Chilton and Beamish 1982; Beamish and McFarlane 1983; Lowerre-Barbieri et al. 1994; Ihde 2000; Sipe and Chittenden 2001).

Our finding that sectioned otoliths are superior disagrees with Barger (1990), who chose whole otoliths over sectioned otoliths for aging Gulf of Mexico bluefish. Barger reported problems with fracturing of otoliths during sectioning and too closely spaced marks on sectioned otoliths. In contrast, we did not find difficulty with close spacing of marks in sectioned otoliths of bluefish from the Chesapeake Bay region, and we found that problems with fracturing were alleviated with sectioning experience. Barger's (1990) report of 70% agreement between whole and sectioned otoliths was similar to our observations. However, he did not indicate the age range examined or report whether his disagreements were systematic or ran-


A
Whole otolith count
95% agreement
32% agreement
45°
B
Scale count
88% agreement
26% agreement
45°
C
Dorsal spine count
68% agreement
28% agreement
45°
Sectioned otolith count

dom, so it is unclear from his study whether whole or sectioned otoliths were better at older ages.

We found whole otoliths to be the second best structure for aging bluefish from the Chesapeake Bay region. Whole otoliths had the second-highest within reader agreement and the highest agreement with sectioned otoliths, especially at younger ages. They were generally easy to read in fish under age 4, and they are probably adequate at these ages. Marks on whole otoliths of older fish were not as clear or as easy to read, however, as those on sectioned otoliths because whole otoliths tended to become increasingly opaque at older ages.

Scales were inferior to both sectioned and whole otoliths for aging bluefish. Marks on scales were often difficult to interpret with objective aging criteria, false marks and regenerated scales were common, and marks at the scale edge were crowded in older fish. As a result, both within- and between-reader agreement, agreement with sectioned otolith age, and confidence scores were undesirably low in scales, especially in fish over age 4. The difficulties we found in using scales to age bluefish generally agree with reports in the literature (Backus 1962; Lassiter 1962; Richards 1976). Richards (1976) found regenerated scales and false annuli to be common, and like Backus (1962), she found that readings varied among scales from the same fish. Lassiter (1962) also reported problems with false annuli on scales and difficulty determining ‘‘the exact number and position of annuli on scales from large fish because of the degree of opacity.’’ Barger (1990) and Chiarella and Conover (1990) are the only investigators to report agreement in scale age readings. Barger (1990) found only 67% between-reader scale agreement, similar to the 62% we found. Chiarella and Conover (1990) reported higher agreement (89%) in repeated scale readings; however, it is not clear what ages they used, and we saw similarly high agreement in scales from younger fish. Chiarella and Conover (1990) reported 93% agreement between scales and sectioned otoliths for fish up to age 6, similar to the 95% agreement we found for ages 0–4. However, they did not examine older fish. Their agreement might have dropped off at older ages because we found only 26% agreement between scales and sectioned otoliths past age 4.

Dorsal spines were inferior to sectioned otoliths, whole otoliths, and scales for aging bluefish. They had the lowest within- and between-reader agreement and the lowest overall confidence scores. Our finding that growth of the vascular core may erode early marks in older fish has also been observed in other species (Hill et al. 1989; Gaichas 1997).

Interpretation of marks on bluefish structures, even sectioned otoliths, was often difficult, as indicated by the lower structure agreement compared with that reported for other more easily aged species, such as summer flounder *Paralichthys dentatus* (Sipe and Chittenden 2001) and spotted seatrout *Cynoscion nebulosus* (Ihde 2000). For example, we found only 86% within- and 83% between-reader agreement for sectioned otoliths in bluefish, whereas spotted seatrout (Ihde 2000) and summer flounder (Sipe and Chittenden, 2001) had 100% and 97% within-reader agreement, respectively, and 100% and 96% between-reader agreement, respectively. The difficulties we encountered with bluefish structures closely resemble those of Spanish mackerel *Scomberomorus maculatus* (Gaichas 1997), another difficult-to-age species that has had problems with structure agreement. Sectioned otoliths, the structure that Gaichas (1997) recommended for aging Spanish mackerel, had only 79% within- and 59% between-reader agreement, the highest values of all the structures examined.

Finally, although we found sectioned otoliths to be the best structure for aging bluefish from the Chesapeake Bay region, our study has not proven their accuracy. However, we feel there is sufficient evidence to recommend that sectioned otoliths replace scales for aging bluefish until validation studies are completed.

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FIGURE 2.—Comparisons of mark counts on sectioned otoliths with mark counts on **(A)** whole otoliths, **(B)** scales, and **(C)** dorsal spine sections. The 45° diagonal line represents 100% agreement. The number of fish is indicated at each symbol. Percent agreement between structures is indicated for younger (≤4 years) and older (>4 years) fish.

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References

Backus, R. H. 1962. Age in a small sample of bluefish (*Pomatomus saltatrix* Linnaeus). Brevoria 159:1–4.

Bagenal, T. B., and F. W. Tesch. 1978. Age and growth. Pages 101–136 i*n* T. B. Bagenal, editor. Methods for assessment of fish production in fresh waters, 3rd edition. Blackwell Scientific Publications, Oxford, UK.

Barger, L. E. 1990. Age and growth of bluefish *Pomatomus saltatrix* from the Northern Gulf of Mexico and U.S. South Atlantic coast. U.S. National Marine Fisheries Service Fishery Bulletin 88:805–809.

Beamish, R. J., and G. A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Transactions of the American Fisheries Society 112:735–743.

Chiarella, L. A., and D. O. Conover. 1990. Spawning season and first-year growth of adult bluefish from the New York Bight. Transactions of the American Fisheries Society 119:455–462.

Chilton, D. E., and R. J. Beamish. 1982. Age determination methods for fishes studied by the groundfish program at the Pacific Biological Station. Canadian Special Publication of Fisheries and Aquatic Sciences 60:1–102.

Diagnostic Instruments, Inc. 1999. User guide to the SPOT RT software. Diagnostic Instruments, Inc., Sterling Heights, Michigan.

Gaichas, S. K. 1997. Age and growth of Spanish mackerel, *Scomberomorus maculatus*, in the Chesapeake Bay region. Master's thesis. College of William and Mary, Williamsburg, Virginia.

Hamer, P. E. 1959. Age and growth studies of the bluefish (*Pomatomus saltatrix* Linnaeus) of the New York Bight. Master's thesis. Rutgers University, New Brunswick, New Jersey.

Hill, K. T., G. M. Calliet, and R. L. Radtke. 1989. A comparative analysis of growth zones in four calcified structures of Pacific blue marlin, *Makaira nigricans*. U.S. National Marine Fisheries Service Fishery Bulletin 87:829–843.

Ihde, T. F. 2000. Biology of the spotted seatrout, *Cynoscion nebulosus*, in the Chesapeake Bay region. Master's thesis. College of William and Mary, Williamsburg, Virginia.

Lassiter, R. R. 1962. Life history aspects of the bluefish, *Pomatomus saltatrix* (Linnaeus) from the coast of North Carolina. Master's thesis. North Carolina State College, Raleigh.

Lowerre-Barbieri, S. K., M. E. Chittenden, Jr., and C. M. Jones. 1994. A comparison of a validated otolith method to age weakfish, *Cynoscion regalis*, with the traditional scale method. U.S. National Marine Fisheries Service Fishery Bulletin 92:555–568.

MAFMC (Mid-Atlantic Fishery Management Council). 1990. Fishery management plan for the bluefish fishery. MAFMC, Dover, Delaware.

Richards, S. W. 1976. Age, growth, and food of bluefish (*Pomatomus saltatrix*) from East-Central Long Island Sound from July through November 1975. Transactions of the American Fisheries Society 105: 523–525.

Sipe, A. M. 2001. Evaluation of opercular bones for aging eight species of Chesapeake Bay fishes. Master's thesis. College of William and Mary, Williamsburg, Virginia.

Sipe, A. M., and M. E. Chittenden, Jr. 2001. A comparison of calcified structures for aging summer flounder, *Paralichthys dentatus*. U.S. National Marine Fisheries Service Fishery Bulletin 99:628–640.

Terceiro, M., and J. L. Ross. 1993. A comparison of alternative methods for the estimation of age from length data for Atlantic coast bluefish (*Pomatomus saltatrix*). U.S. National Marine Fisheries Service Fishery Bulletin 91:534–549.

Wilk, S. J. 1977. Biological and fisheries data on bluefish, *Pomatomus saltatrix* (Linnaeus). NOAA Technical Report NMFS 11.

Zar, J. H. 1996. Biostatistical analysis. Prentice Hall, Upper Saddle River, New Jersey.