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A Comparison Of Calcified Structures For Aging Summer Flounder, Paralichthys Dentatus

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Abstract–Calcified structures of summer flounder, *Paralichthys dentatus*, were evaluated to identify the best age determination method. Scales, the currently preferred structure, were compared with opercular bones and to right and left whole and sectioned otoliths for ages 0 to 10. All structures showed concentric rings that were interpreted as annual; however structures differed greatly in the clarity of their presumed annual marks. Right and left otoliths generally gave the same age, although they differed in the clarity of marks. Sectioned otoliths, particularly right ones, were the best aging structure. Right sectioned otoliths consistently showed the clearest marks and had the highest confidence scores, lowest reading times, and highest agreement within and between readers, 97% and 96%, respectively. Left sectioned otoliths took twice as long to prepare and were more difficult to interpret than right sectioned otoliths. Whole otoliths were the second best structure and were adequate to age 4 or 5, after which sectioning greatly improved the clarity of marks. Scales were inferior to, and often did not give the same age readings as, whole and sectioned otoliths. Compared with otoliths, scales tended to overage at younger ages and to underage at older ages. Opercular bones were undesirable for aging summer flounder. They were often unclear and inconsistent, and they had the lowest confidence scores, the highest reading times, and only 46% within-reader agreement. A major source of disagreement in scale and otolith age readings was the presence of an early, presumably false, mark on some structures. We compare the formation of this early mark in summer flounder with early mark formation on otoliths of Atlantic croaker, a species with similar life history traits.

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A comparison of calcified structures for aging summer flounder, *Paralichthys dentatus**

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The summer flounder, *Paralichthys dentatus*, ranges from Nova Scotia to Florida, although it is most abundant from Massachusetts to North Carolina (Ginsburg, 1952; Leim and Scott, 1966; Gutherz, 1967). In regions of high abundance, it is one of the most important commercial and recreational fishes on the Atlantic coast (MAFMC, 1987). In the Chesapeake Bay region, for example, summer flounder support an extensive recreational fishery from about March to November, when they are present in the lower portions of the Chesapeake Bay and in coastal waters (Hildebrand and Schroeder, 1928; MAFMC, 1987; Desfosse, 1995). They then support a strong commercial fishery during the fall and winter, when they move offshore to the continental shelf (Ginsburg, 1952; Bigelow and Schroeder, 1953; Poole, 1962; MAFMC, 1987).

Many studies have reported difficulties with the structures used for age determination of summer flounder. Prior to about 1980, whole left otoliths were the most commonly used structure (Poole, 1961; Eldridge, 1962; Smith and Daiber, 1977; Powell, 1982). However, there were disagreements over the location and interpretation of the first presumed annual mark (Poole, 1961; Eldridge, 1962; Smith and Daiber, 1977), largely a result of uncertainties about first year growth rates. This and other problems with whole otoliths (summarized in Smith et al., 1981) prompted a comparison of age determination structures by Shepherd (1980), who reported that presumed annual marks were more distinct on scales than on whole otoliths. Consequently, scales became the preferred structure for aging summer flounder (Smith et al., 1981; Dery, 1988; Almeida et al., 1992). More recently, Szedlmayer et al. (1992) examined first year growth rates to resolve the location and interpretation of the first mark on whole otoliths, but scales have remained the preferred structure (Bolz et al., 2000).

Difficulties have also been reported in using summer flounder scales (Dery, 1988; Desfosse, 1995; Bolz et al., 2000). Desfosse (1995) used marginal increment analysis to validate scales for ages 1 to 3. He reported only 46% within-reader agreement past age 4, however, indicating that marks on scales are not very distinct at older ages. He attributed disagreements to false or indistinct annuli and to crowding of annuli at the scale edge in older fish. Most recently, Bolz et al. (2000) reported only 53% agreement for ages 1 to 5 in a between-agency exchange of scales, with agreement increasing to only 83% after they resolved as many disagreements as possible. They attributed most of the remaining disagreements to the choice of a first annual mark and to differing opinions on what constituted a false mark on scales.

A reexamination of calcified structures for aging summer flounder is needed, given their economic importance and the reported difficulties in

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age determination with whole otoliths and scales. Previous studies have never evaluated sectioned otoliths in summer flounder, even though sectioned otoliths have often proven a superior structure in other species, especially at older ages when scales and other structures can underage fish (Beamish and McFarlane, 1983). Further study is especially needed because the location of the first mark on otoliths has recently been determined (Szedlmayer et al., 1992). In addition, no work has been done to determine if right-left differences in the location of the focus result in differences in age determination.

The main objective of our study was therefore to evaluate and compare whole otoliths, sectioned otoliths, scales, and opercular bones for aging summer flounder. We included opercular bones because many studies, on a variety of species, have found them to be superior to other structures and to have very distinct and easy to read marks (for examples, see LeCren, 1947; Donald et al., 1992; Hostetter and Munroe, 1993). A second objective was to compare right and left otoliths for potential differences in age based on differences in the location of the focus. Calcified structures were evaluated in terms of preparation and reading times, confidence in presumed annual mark clarity, agreement between repeated age readings, structure growth with fish growth, age agreement between different structures of the same fish, and increases in the number of presumed annual marks with structure size and fish size. Finally, we discuss the formation of early, presumably false, marks on summer flounder otoliths and scales that resulted in difficulties in age interpretation

Methods

Sample collection

To minimize difficulties interpreting marks on the edge of the structures, collections of summer flounder were made far from the time of presumed annual mark formation, which occurs in May and June on the scales of Chesapeake Bay summer flounder (Desfosse, 1995). Summer flounder were collected from commercial fisheries in the Chesapeake Bay region from September through November of 1998 (n=165). Additional juvenile fish (n=11) were collected by the Virginia Institute of Marine Science juvenile bottom trawl survey in October of 1998 in the lower Chesapeake Bay and James River.

Fish were processed for total length (TL), total weight (TW), and sex, and the calcified structures were removed as follows. Both saggital otoliths were removed, wiped clean, and stored dry in tissue culture cell wells. Scales were removed from just above the lateral line anterior to the caudal peduncle (Shepherd, 1980; Dery, 1988) and stored in coin envelopes. Both opercular bones were removed according to the methods of LeCren (1947), stored in coin envelopes, and frozen.

The collection of summer flounder was stratified into six length-based categories of 100 mm each to include as many age groups as possible in the final study sample. A random sample of 15 fish was then chosen from the first five categories. The last category included the six largest fish, all of which were used in the comparison, for a total of 81 fish. All calcified structures in the final study sample were assigned random numbers before preparation and aging. Summer flounder in the final study sample ranged in size from 209 to 758 mm TL and from 80.8 to 7304.6 g TW and in age from 0 to 10 years (determined from sectioned otoliths, as reported in this study).

Preparation of calcified structures for age determination

Whole otoliths were examined in water on a dark background with reflected light at 120 to 240× magnification. Thin opaque bands, which appeared white under reflected light, were presumed to represent annual marks (Fig. 1A). Two counting paths were used for mark enumeration. The primary counting path was from the focus to the anterior margin of the otolith. The secondary counting path, used to verify the primary counting path reading, was from the focus to the posterior margin of the otolith. With calipers to 0.05 mm, whole otolith total length (WOTL) was measured as the largest distance from the anterior to the posterior edge and whole otolith radial length (WORL) was measured from the center of the focus to the tip of the anterior edge. A paired sample t-test was used to test for right-left differences in WORL.

After all whole otolith readings were made, right and left otoliths were mounted sulcal groove down onto cardboard with crystal bond adhesive and sectioned transversely through the focus with a variable speed Beuhler Isomet saw. The resulting sections, about 0.5 mm thick, were mounted on clear glass slides and immersed in crystal bond. Sections were viewed with transmitted light and bright field at 240× magnification. Thin opaque bands, which appeared dark with transmitted light, were presumed to represent annual marks (Fig. 1B) and were counted along the ventral side of the sulcal groove. Sectioned otolith radial length (SORL) was measured to 0.001 mm along the ventral arm of the sulcal groove from the center of the focus to the otolith edge by using a compound video microscope with the Optimas image analysis system (Media Cybernetics, 1999). Broken otoliths were not measured if they were fractured along the focus. A paired sample t-test was used to test for right-left differences in SORL.

Opercular bones were prepared according to the methods of LeCren (1947). Briefly, they were soaked in cold tap water for several minutes to thaw and to partially loosen surrounding skin, then soaked for 1 minute in simmering water, after which the skin was easily removed with a toothbrush. The opercular bones were then rinsed with cold tap water and air-dried. Opercular bones were examined dry with transmitted light and in water with reflected light on a dark background. Presumed annual marks (Fig. 1C) were defined as sharp transitions from relatively narrow translucent zones to relatively wide opaque zones that were continuous from the anterior to the posterior margin of the bone (Bagenal and Tesch, 1978; Hostetter and Munroe, 1993). Translucent zones appeared white under

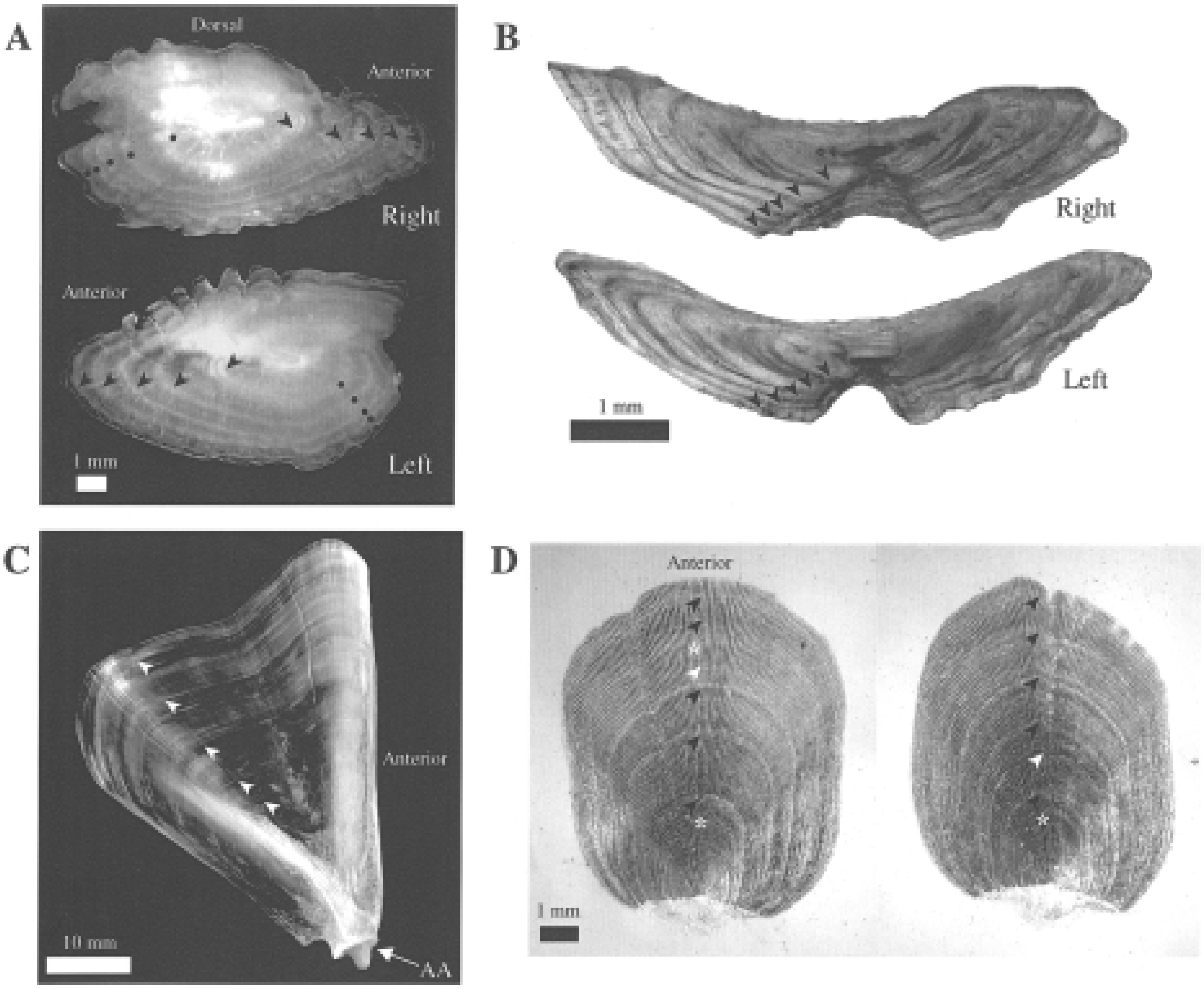

Figure 1

Marks on calcified structures taken from a 5-year-old (determined from sectioned otoliths) female summer flounder, TL = 687 mm, collected in mid-January. Arrows indicate individual marks counted (as described in the "Methods" section). (**A**) Whole otoliths, viewed with reflected light on a black background. Arrows indicate presumed annual marks along the primary counting path; dots indicate presumed annual marks along the secondary path. Whole otolith radial lengths were measured along the primary counting path. (**B**) Transverse otolith sections, viewed in transmitted light. Sectioned otolith radial lengths were measured along the counting path (indicated by arrows) from the center of the focus to the edge along the ventral arm of the sulcal groove. (**C**) Right opercular bone, viewed with reflected light on a black background. AA = articular apex. (**D**) Scale impressions, viewed in transmitted light. White arrows indicate marks that appear on only one of the scales. Asterisks indicate probable false marks. Both scales have a probable false mark prior to the first mark counted.

transmitted light and dark under reflected light, whereas opaque zones appeared dark under transmitted light and white under reflected light. The first presumed annual mark was defined as the first opaque zone after the first translucent zone, where the first translucent zone occupied the central focal area of the opercular bone. Both bones were examined, and the one with the clearest marks was used for aging. Opercular bone radial length (OpRL) was measured to 0.05 mm from the center of the articular apex to the anterior margin edge with calipers.

Scales were soaked in water until flexible and brushed gently with a soft bristle toothbrush. Then 5 or 6 clean, symmetrical, unregenerated scales were dried, taped to an acetate sheet, inserted between two new acetate sheets, and pressed in a Carver laboratory scale press for 2 minutes at 15,000 pounds of pressure and 60°C. Scale impressions were read with a Bell-Howell R753 microfiche reader at 20× and 32×. Presumed annual marks were identified with standard scale reading criteria as described in Smith et al. (1981), Dery (1988), and Almeida et al. (1992). Briefly, readers enumerated marks (Fig. 1D) that exhibited “cutting over” in both lateral fields of the scale that was accompanied by a clear narrow zone in the anterior portion of the scale. Scale radial length (ScRL) was measured to 0.001 mm from the center of the focus to the anterior edge of the scale by using a compound video microscope with the Optimas image analysis system (Media Cybernetics, 1999).

Evaluation of calcified structures

Each structure was examined for age by two readers—twice by reader 1 and once by reader 2. Structures were read in a randomly selected order with no knowledge of fish size or collection date. Ages were assigned on the basis of presumed annual mark counts. Different structures from the same fish were read independently, including right and left otoliths, and at least one week separated the first and second readings of the same structure.

Preliminary evaluations of structures included preparation times, reading times, confidence in the clarity of presumed annual marks, growth of the structures with size of the fish, and agreement in repeated age readings of the same structure (precision). Structures judged acceptable based on those criteria were then evaluated further for agreement in age readings between different structures from the same fish and to see if the number of presumed annual marks increased with structure size and fish size. Our preliminary evaluation indicated otoliths and scales to be superior to opercular bones; therefore opercular bones were not evaluated further.

Preparation time, a measure of the processing efficiency of a structure, was evaluated as the time taken to prepare structures for reading. Clarity of presumed annual marks on a structure was evaluated using both reading times and confidence scores. Reading time was measured as the time taken to read a given structure in an individual fish. Confidence scores, expressed on a scale of 1 (low) to 5 (high), were assigned by the reader to each reading based on the clarity of the marks. Differences in confidence scores between structures were tested at $\alpha = 0.05$ by using the normal approximation to the Mann-Whitney test for ordinal data (Zar, 1996).

The assumption that structure growth is directly related to fish growth was evaluated using regression analysis (Zar, 1996). Structure sizes (ScRL, OpRL, WOTL, WORL, SORL) were regressed on fish TL to determine if the relationships were significant and increasing. Sample sizes varied in these regressions, and in regressions of the number of presumed annual marks on structure size described below because some structures were broken in preparation and could not be measured.

Precision in age determinations for a given structure was evaluated using simple percent agreement in repeated readings within and between readers. Within-reader agreement compared the first and second readings by reader one, and between-reader agreement compared the first readings of each of the two readers. Reader comments on structure features were evaluated to determine the proximal causes of disagreements.

Scales that disagreed in the initial two readings by reader 1 were reread independently a third time by reader 1 to reach a consensus for use in between-structure comparisons. Likewise, right and left otoliths that disagreed in the initial two readings by reader 1 were read a third time to reach a consensus. Structures that showed no agreement in three readings (1 of 81 for scales, 1 of 81 for sectioned otoliths) were not included in between-structure comparisons.

Agreement in presumed annual mark counts between different structures of an individual fish was evaluated by using simple percent agreement between structures and simple linear regression procedures. For the regressions, ages determined by one structure were regressed on ages determined by another structure, and the slope of the regression line was tested to see if it differed significantly from one. A slope of one implies that $y = x$ and that the two structures give the same age. For each regression, we used as the x-variable the structure judged to be superior in the preliminary evaluations.

The assumption that the number of presumed annual marks on a structure is directly related to structure size and to fish size was evaluated using regression analysis (Zar, 1996). The number of presumed annual marks on a structure was regressed on structure size (ScRL, WOTL, WORL, SORL) and on fish TL to determine if the relationships were significant and increasing.

Results

Comparative appearance of calcified structures

All four calcified structures showed concentric marks that were interpreted as annual (Fig. 1). However, these structures differed greatly in the clarity of presumed annual marks.

Presumed annual marks on both whole and sectioned otoliths (Fig. 1, A and B) were typically clear, consistent, and easy to interpret, especially for sectioned otoliths. The right-left difference in the location of the focus had moderate effects on mark clarity for both whole and sectioned otoliths, as described below. Whole otolith marks were most easily read at younger ages, but age had little effect on sectioned otolith mark clarity. The few disagreements in otolith ages were primarily caused by an early, presumably false, mark that often occurred prior to the first presumed annual mark (Fig. 2). This early mark appeared as a thin opaque band close to, but distinct from, the focus and was found on both young (Fig. 2A) and older (Fig. 2B) fish. We tried not to count this early mark in our age readings, because it did not occur consistently in all fish. Finally, only one otolith of 81 pairs was poorly calcified and unable to be read whole, although its age was easily determined upon sectioning.

Presumed annual marks on opercular bones (Fig. 1C) were fairly clear in some fish, but they were more often poorly defined, inconsistent, and difficult to follow across the structure, making age interpretation difficult and highly subjective. Opercular bones commonly exhibited unclear transitions from translucent to opaque zones; the first one or two marks were particularly difficult to distinguish, even on young fish. Zone transitions were often easier to interpret towards the edge of the structure in older fish, although this too varied greatly from fish to fish. The example in Figure 1C is unusually clear and easy to read.

Presumed annual marks on scales (Fig. 1D) were clearer than those on opercular bones, but they still required much subjective interpretation. Figure 1D shows some of the common problems encountered with scales, including presumably false marks (asterisks) and marks that were present on only some scales from the same fish (white arrows). In addition, many fish had regenerated, asymmetrical, or otherwise damaged scales, making it difficult and time-consuming to choose acceptable scales to press. For example, about 20 scales were pressed in order to obtain two scales that were adequate to show in Figure 1D. Interpretation of age from scales of older fish was extremely difficult because marks at the scale edges were often obscured or crowded together, particularly in the narrow lateral fields. Finally, a major source of disagreement in age determination from scales resulted from an early, presumably false, mark that often occurred prior to the first presumed annual mark (Fig. 1D, asterisk). Because this early mark did not appear consistently in all fish or even on several scales from the same fish, we tried not to count it in our age readings.

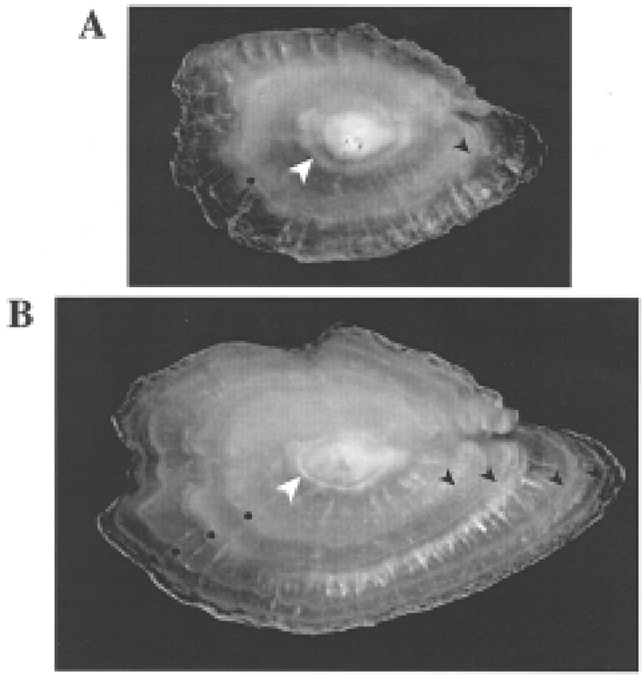

Figure 2

Right whole otoliths showing an early, presumably false, mark. (**A**) is from a 299-mm-TL age-1 fish collected in September, and (**B**) is from a 442-mm age-4 fish collected in October. White arrows point to the early marks. Black arrows indicate primary counting path (anterior field), dots indicate secondary counting path (posterior field).

Preparation times, reading times, and confidence in clarity of marks

Preparation times were short and reasonable for all structures, at less than 15 minutes per fish. Whole otoliths took by far the shortest time because no preparation was required before reading (Table 1). Sectioned right otoliths and opercular bones required 4 to 6 minutes to prepare, whereas scales and sectioned left otoliths took much longer to prepare, about 11 and 14 minutes, respectively. Left sectioned otoliths took much longer to prepare than right sectioned otoliths primarily because they broke much more frequently during sectioning.

Reading times were short and reasonable for all structures, at less than three minutes per fish. Sectioned right otoliths had by far the shortest reading time, at only 0.27 minutes per fish (Table 1). Whole otoliths and sectioned left otoliths had the next shortest reading time, at only about 0.4 to 0.6 minutes per fish. Scales (1.2 min) and opercular bones (2.4 min) both required much more reading time than otoliths, indicating that otoliths could be aged more easily.

Reader confidence scores varied greatly between structures. Sectioned otoliths had by far the highest confidence scores, with values of 4.9 and 4.8 for the right and left, respectively (Table 1). Whole otoliths had somewhat lower confidence scores, with values of 4.1 and 3.8 for the right and left, respectively. Confidence scores were much lower for scales (3.2) and especially for opercular bones (2.3), indicating that these structures were not as easily interpreted. All confidence scores were significantly different from one another (Z=2.10 to 4.18; P<0.0001 to 0.013; individual values not reported).

Regression of structure size on fish size

All calcified structures grew in size as summer flounder body length grew, indicating that each structure could be useful for back-calculation studies. All regressions of structure size on total length were significant at $P < 0.001$, and all slopes were positive (Table 2). All regressions were strong and explained much of the variation in structure size, generally 90% or more, with coefficient of determination values (100 r^2) ranging from 72% to 98%. Values for 100 r^2 were less than 91% only for right and left sectioned otoliths, which were 72% and 85%, respectively.

Agreement in age determinations for the same structure

Agreement (precision) between repeated age readings varied greatly between calcified structures. Precision by the same reader was highest by far (95% to 97%) for sectioned right and left otoliths and left whole otoliths (Table 3). Precision was somewhat lower in right whole otoliths (89%) than in

Table 1

Average preparation times (min), reading times (min) ± standard error (SE), and confidence scores (±SE) for summer flounder calcified structures.

Structure	Preparation time	Reading time	Confidence score
Opercular bones	4.63	2.43 ±0.20	2.31 ±0.16
Scales	10.50	1.20 ±0.13	3.21 ±0.15
Sectioned otoliths			
Right	5.86	0.27 ±0.04	4.91 ±0.04
Left	13.93	0.57 ±0.09	4.75 ±0.05
Whole otoliths			
Right	0.00	0.45 ±0.06	4.10 ±0.11
Left	0.00	0.41 ±0.04	3.84 ±0.10

Table 2

Regression statistics for relationships between structure size and summer flounder total length (TL). Structure abbreviations are defined in the "Methods" section of the text. n = sample size. All regressions were significant at $P < 0.001$.

Structure	Equation	n	100 r^2
Opercular bones	$OpRL = -2.280 + 0.0772\ TL$	66	98
Scales	$ScRL = -0.348 + 0.0126\ TL$	81	93
Sectioned otoliths			
Right	$SORL = -0.015 + 0.0027\ TL$	66	85
Left	$SORL = 0.015 + 0.0018\ TL$	47	72
Whole otoliths			
Right	$WORL = 0.642 + 0.0089\ TL$	76	91
Left	$WORL = 0.601 + 0.0111\ TL$	77	93
Right	$WOTL = 1.280 + 0.0164\ TL$	76	94
Left	$WOTL = 1.530 + 0.0156\ TL$	77	91

Table 3

Average percent agreement, within and between readers, for presumed annual mark counts on summer flounder calcified structures.

Structure	Within reader	Between reader
Opercular bones	46	—
Scales	80	58
Sectioned otoliths		
Right	97	96
Left	95	88
Whole otoliths		
Right	89	86
Left	97	87

left whole otoliths; however this could be attributed to the reader learning to use reflected lighting more effectively during the second reading, because 7 of the 9 consensus readings for right otoliths agreed with the second reading. Within-reader agreement was lower with the use of scales (80%), but precision varied with age. Agreement in repeated scale readings was actually high for ages 0 to 4 (92%, n=52), but it decreased to only 59% for fish over age 4 (n=29). Precision was lowest by far in opercular bones (46%), where there were no patterns in agreement by age. Because opercular bones showed the lowest precision and the poorest mark clarity, we did not include them in further evaluations.

Agreement in age determinations between readers also varied greatly among calcified structures. Precision between readers was highest by far (96%) for right sectioned otoliths (Table 3). Agreement was somewhat lower (86% to 88%) for left sectioned otoliths and whole otoliths. Agreement was lowest by far for scales (58%), reflecting the overall poor clarity of marks and the resulting subjectiveness in scale age readings compared with otolith age readings.

Comparison of right and left otoliths

Differences in right and left radial lengths were observed for both whole and sectioned otoliths. The right radial length was significantly shorter than the left in whole otoliths (paired t=17.59, df=73, P<0.0001; Fig. 1A). However, for sectioned otoliths, the right radial length was significantly longer than the left (paired t =–11.72, df=43, P<0.0001; Fig. 1B) because the right otolith is thicker at the focus, where the transverse cross section was taken.

Right and left whole otoliths generally gave the same age readings. Reader one had high age agreement between right and left whole otolith readings (96%), and the null hypothesis that the slope of the line equals one was not rejected (P=0.077, Fig. 3A).

Although right and left whole otoliths generally indicated the same age, they differed in mark clarity. When the posterior field (secondary counting path) was used to verify or determine the number of presumed annual marks, the right otolith was generally much easier to read than the left because of the greater distance between the focus and the posterior margin on the right otolith (Fig. 1A). This greater distance made the marks further apart and more easily distinguishable on the right than on the left otolith. The difference in mark clarity was greatest for older fish and was also reflected in significantly higher confidence scores for the right whole otolith than for the left (Table 1).

Right and left sectioned otoliths also generally gave the same age readings. Reader one had high age agreement between right and left sectioned otolith readings (94%), and the null hypothesis that the slope of the line equals one was not rejected (P=0.393, Fig. 3B).

Although right and left sectioned otoliths generally gave the same age, presumed annual marks were usually clearer and easier to interpret on the right otolith. Right sectioned otoliths had a much longer counting path and were therefore easier to age than left sectioned otoliths, where the marks were more crowded and less clearly defined (Fig. 1B). This difference was also reflected in higher confidence scores and lower reading times for the right sectioned otolith than for the left (Table 1).

Comparison of different calcified structures from the same fish

Whole and sectioned otoliths generally gave the same age readings. The number of presumed annual marks on whole and sectioned otoliths showed high agreement (95%), with 100% agreement for fish under age 4 (Fig. 4A). In addition, the null hypothesis that the slope of the line equals one was not rejected (P=0.901).

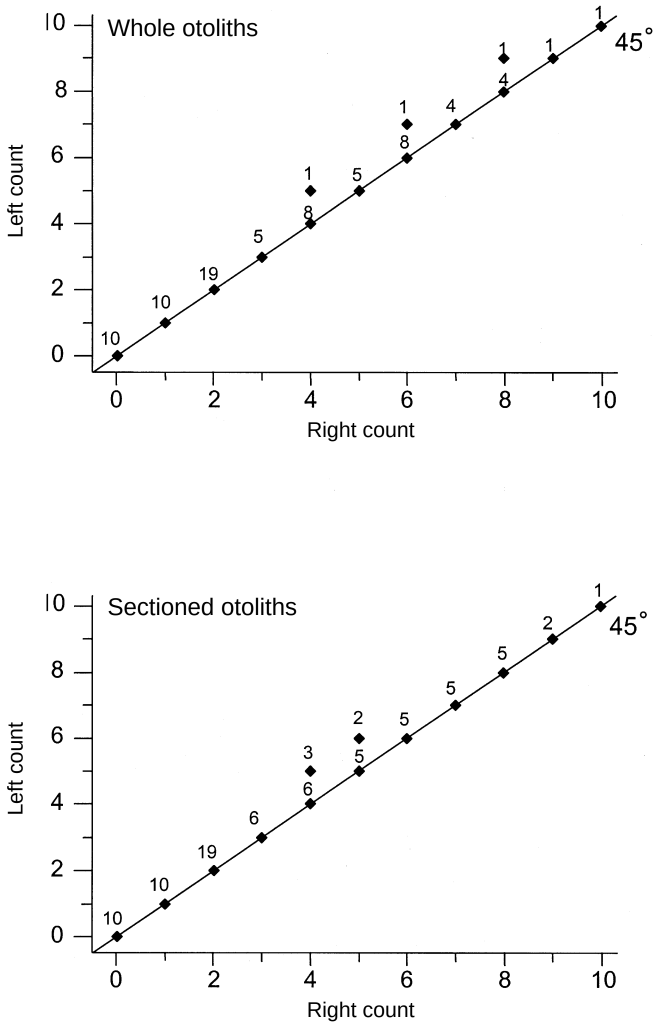

Figure 3

Comparisons of presumed annual mark counts on the left otolith with mark counts on the right otolith for whole otoliths and sectioned otoliths in summer flounder. The 45° diagonal line represents 1:1 agreement. The number of fish is indicated at each symbol.

Although whole and sectioned otoliths generally provided the same age, presumed annual marks were often clearer on sectioned otoliths than on whole ones, especially in older fish, where crowding of marks at the edge of whole otoliths became a problem. This observation is supported by the much higher confidence scores for sectioned otoliths (Table 1). As a specific example, the oldest fish in the comparison showed very clear marks and was aged 10 in every reading using both right and left sectioned otoliths (Fig. 5A), and all confidence scores were 5. Marks were less clear on the whole otolith (Fig. 5B), however, with between 8 and 10 marks counted in different readings, and an average confidence score of only 2.5. In general, the use of sectioned otoliths appeared to greatly increase mark clarity in fish over age 4 or 5.

Scales and sectioned otoliths often did not give the same age readings. Agreement in the number of presumed annual marks on scales and sectioned otoliths was undesirably low, at only 80% (Fig. 4B). In addition, the null hypothesis that the slope of the line equals one was rejected (P=0.047). Scales tended to overage compared with sectioned otoliths in fish age 4 and younger, but to underage in fish older than age 4. Agreement between scales and sectioned otoliths was fairly high for ages 0 to 4 (86%, n=56) but decreased to only 65% in fish over age 4 (n=23).

Scales and whole otoliths often did not give the same age readings. Agreement in the number of presumed annual marks on scales and whole otoliths was also undesirably low, at only 76% (Fig. 6). In addition, the null hypothesis that the slope of the line equals one was again rejected (P=0.039). As with sectioned otoliths, scales tended to overage compared with whole otoliths in fish age 4 and younger and to underage in fish older than age 4. Agreement between whole otoliths and scales was fairly high for ages 0 to 4 (85%, n=53) but decreased to only 56% in fish over age 4 (n=25).

Increase in number of marks with structure size and fish size

Mark counts on calcified structures increased as structure size and fish size increased, indicating that each structure tested could be useful in age determination. All regressions of mark counts on structure size were significant at P<0.001, and all slopes were positive (Table 4). Regressions were generally strong and explained much of the variation in mark counts because 100 r^2 values were high, generally from 80% to 86%. Values for 100 r^2 were lowest for left sectioned otolith radius and scale radius, at 67% and 73%, respectively. Likewise, all regressions of mark counts on fish size were significant at $P < 0.001$, and all slopes were positive (Table 5). All regressions were again strong, with 100 r^2 values from 83% to 86%.

Discussion

Comparative evaluation of sectioned otoliths

Our findings indicate that sectioned otoliths are the best structure for aging summer flounder over the age range 0 to 10 years. Sectioned otoliths had the shortest reading times, the highest confidence scores, the highest within- and between-reader agreement, and they were consistently clearer and easier to read than whole otoliths, scales, and opercular bones. These findings are new for summer flounder because no published studies have used sectioned otoliths to age this species. These findings generally agree, however, with many studies on other species that have found sectioned otoliths to be the best aging structure (for examples, Beamish, 1979; Chilton and Beamish, 1982; Beamish and McFarlane, 1983; Lowerre-Barbieri et al., 1994).

Right sectioned otoliths were generally superior to left sectioned otoliths. Although we found high agreement in age between right and left sectioned otoliths, right otoliths were much easier to prepare, and they had a larger counting path, which made it easier to identify the marks, resulting in shorter reading times, higher confidence scores, and higher reader agreement.

Although we have found sectioned otoliths to be the best structure for determining the age of summer flounder, our studies have not proven their accuracy. To do so would require known-age methods or at least marginal increment methods. However, until validation is done, we feel there is sufficient evidence to recommend that sectioned otoliths replace the current practice of using scales for aging summer flounder.

Comparative evaluation of whole otoliths

Our findings indicate that whole otoliths are the second best structure for aging summer flounder over the age range of 0 to 10 years. Whole otoliths had no preparation time and had the second shortest reading times, the second highest confidence scores, the second highest within- and between-reader agreement, and the highest agreement with sectioned otoliths. Whole otoliths were generally easy to read in fish less than age 4 or 5, and we feel they are adequate for these younger ages, especially in large-scale production aging where preparation time is important.

We found that the right whole otolith was often easier to read than the left when the secondary counting path was used. Therefore, although former studies have used the left whole otolith only (Poole, 1961; Eldridge, 1962; Smith and Daiber, 1977; Powell, 1982), we suggest that the right should be included in future work.

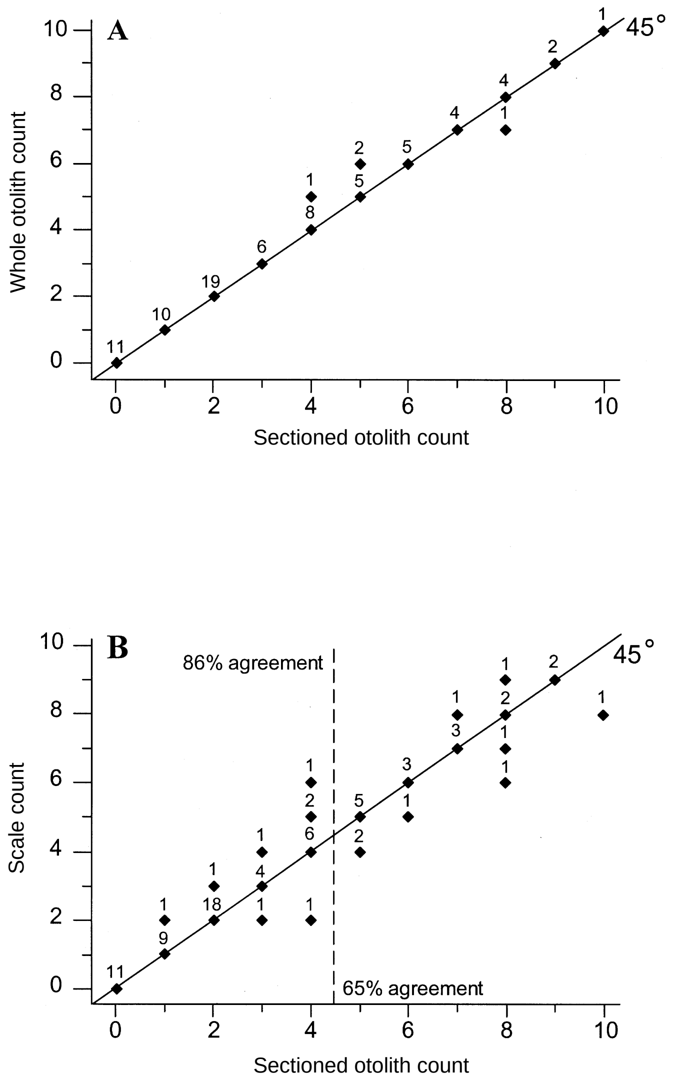

Figure 4

Comparisons of presumed annual mark counts on whole otoliths (**A**) and scales (**B**) with mark counts on sectioned otoliths in summer flounder. The 45° diagonal line represents 1:1 agreement. The number of fish is indicated at each symbol.

Our findings on preparation and reading times, confidence scores, within- and between-reader agreement and agreement with sectioned otoliths are generally new because the literature has not reported detailed evaluations of whole otoliths in summer flounder. Given our findings, we do not agree with the current preference for using scales rather than whole otoliths in summer flounder. Indeed, we disagree with the original reasons for rejecting otoliths, which included 1) poor calcification and poor contrast between opaque and translucent zones (Shepherd, 1980; Smith et al., 1981; Dery, 1988), 2) obscurement of the first mark as the fish ages (Powell, 1982), 3) deviation from the generalized pattern of opaque and translucent zone for-

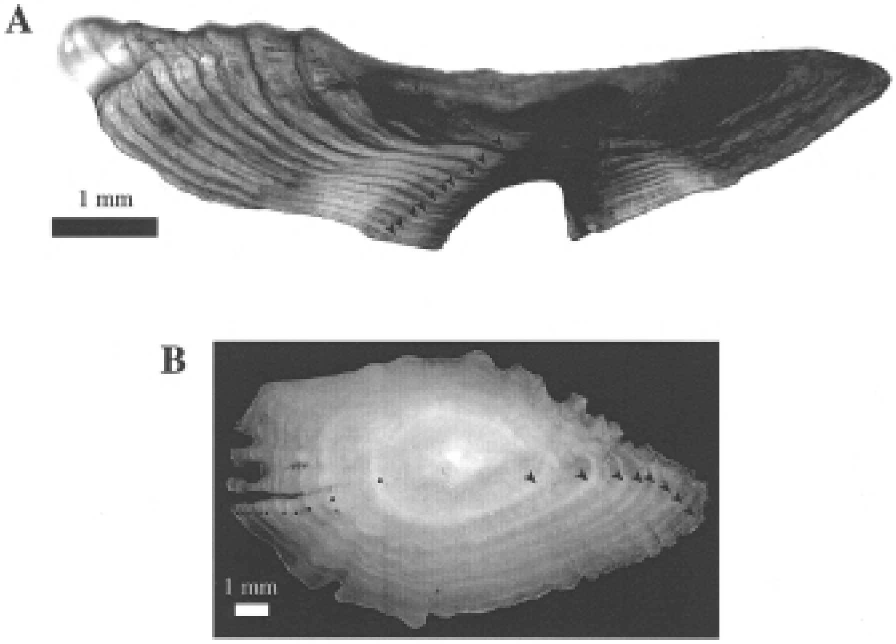

Figure 5

Right sectioned (**A**) and whole (**B**) otolith from a female summer flounder, 10 years old (determined from sectioned otoliths) and 758 mm TL, collected in November. Arrows on the sectioned otolith indicate presumed annual marks. On the whole otolith, arrows indicate primary counting path (anterior field), dots indicate secondary counting path (posterior field). Ten marks are visible in the posterior field of the whole otolith, but only eight marks are visible in the anterior field.

Table 4

Regression statistics for relationships between the number of marks (*Marks*) and calcified structure size for summer flounder. Structure abbreviations are defined in the "Methods" section of the text. n = sample size. All regressions were significant at $P < 0.001$.

Structure	Equation	n	100 r^2
Scales	$Marks = -2.64 + 1.080\ ScRL$	80	73
Sectioned otoliths			
Right	$Marks = -3.39 + 5.424\ SORL$	65	80
Left	$Marks = -3.36 + 6.996\ SORL$	46	67
Whole otoliths			
Right	$Marks = -4.56 + 1.664\ RWOR$	75	85
Left	$Marks = -4.47 + 1.367\ LWOR$	76	86
Right	$Marks = -4.80 + 0.919\ RWOT$	75	86
Left	$Marks = -4.80 + 0.934\ LWOT$	76	82

Table 5

Regression statistics for relationships between the number of marks (*Marks*) on calcified structures and summer flounder total length (*TL*). All regressions were significant at $P < 0.001$, and sample sizes were 80 fish.

Structure	Equation	100 r^2
Scales	$Marks = -3.69 + 0.0151\ TL$	83
Sectioned otoliths	$Marks = -3.86 + 0.0155\ TL$	85
Whole otoliths	$Marks = -3.90 + 0.0157\ TL$	86

mation in temperate fishes (Smith et al., 1981), and 4) a narrow opaque zone as compared to the translucent zone (Smith et al., 1981). We address these issues in turn below.

We rarely observed poor calcification or poor contrast between opaque and translucent zones of whole otoliths. Rather, our procedures gave good contrast between opaque and translucent zones, so that we had high confidence in our age readings. In addition, we found only one otolith of 81 pairs to be poorly calcified. This otolith was easily aged once it was sectioned, and its pair was not poorly calcified and was aged with high confidence.

We saw little evidence that the first mark becomes obscured at older ages on whole otoliths, as indicated by our high agreement between whole and sectioned otoliths. The hypothesis that the first mark becomes obscured was based on overlap in back-calculated sizes at the second and third marks on whole otoliths (Powell, 1982). However, size in any year class can vary greatly because summer flounder spawn over a protracted season (Smith, 1973; Morse, 1981; Able et al., 1990). Therefore, fish in adjacent year classes can be expected to overlap in size, and Powell's results do not necessarily mean that the first mark becomes obscured with age.

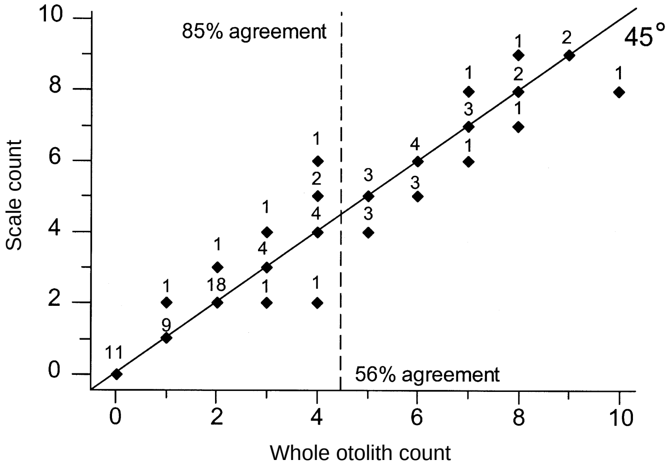

Figure 6

Comparison of presumed annual mark counts on scales and whole otoliths in summer flounder. The 45° diagonal line represents 1:1 agreement. The number of fish is indicated at each symbol.

Smith et al. (1981) reported that summer flounder otoliths deviated from the general pattern of opaque and translucent zone formation seen in other temperate fishes and suggested that opaque zones formed in fall–winter, the reverse of the usual spring–summer formation in other temperate species. We saw no evidence of this reversal. Our fish were collected from October through December, so we should have observed opaque edges on the otolith if the timing of mark formation were reversed from other temperate fishes. Instead, we observed relatively wide translucent zones on the otolith edges. In addition, other studies have not found a reversal in the time of mark formation (Poole, 1961; Powell, 1982; Wenner et al., 1990), and Desfosse (1995) found that opaque zones appeared to form on whole otoliths at approximately the same time as scale marks (May through July). Finally, Smith et al. (1981) presented no data to support their hypothesis that opaque zones formed in the fall and winter. Indeed, their Figure 5 shows an opaque edge on a whole otolith from a summer flounder captured in June.

In agreement with studies in other species (see references below), we found the translucent zone to be wider than the opaque zone on summer flounder otoliths. Smith et al. (1981) felt this was an anomalous occurrence and used it to reject whole otoliths. We disagree with their analysis, however, because many other fishes in our study area, including Atlantic croaker (Barbieri et al., 1994a), weakfish (Lowerre-Barbieri et al., 1994), and Spanish mackerel (Gaichas, 1997) have otoliths with a wide translucent zone and a narrow opaque zone. Such a pattern reflects the fact that opaque zones form over a short time period in these species: April–May in Atlantic croaker and weakfish (Barbieri et al., 1994a; Lowerre-Barbieri et al., 1994) and May–June in Spanish mackerel (Gaichas, 1997). In addition, although the sample size was limited (*n*=93), Desfosse (1995) found evidence, using marginal increments, that opaque zone formation on summer flounder otoliths occurs over a similarly short time period (May to July). Finally, regardless of whether opaque zones are narrower or wider than translucent zones, otoliths can be used for age determination if the mark can be proven annual.

Comparative evaluation of scales

Our findings indicate that scales are inferior to, and much less desirable than, both sectioned and whole otoliths for aging summer flounder. Scales had significantly lower confidence scores and much higher reading times than sectioned and whole otoliths because marks on scales were often difficult to interpret using objective aging criteria. False marks were common, and different scales from the same fish often indicated different ages. As a result, both within- and between-reader percent agreement and agreement with whole and sectioned otolith age were undesirably low in scales, especially in fish over age 4. We feel that scales should not be used for aging summer flounder if otoliths, especially sectioned otoliths, are available.

The difficulties we found with summer flounder scales generally agree with reports in the literature. Dery (1988), Desfosse (1995), and Bolz et al. (2000), for examples, have reported similar problems interpreting scale marks. Like us, Desfosse (1995) found low within-reader scale agreement (only 46%) in fish over age 4. Desfosse (1995) reported high agreement between scales and whole otoliths (98%) for ages 0 to 5, much higher than the 85% agreement we found for ages 0 to 4. However, 90% of his fish (*n*=170) were ages 0 to 2 and only one was age 5, a likely explanation for his high percent agreement. Shepherd (1980) reported high agreement (91%) between scales and whole otoliths for moderately old fish (ages 4 to 6), but his sample size was only 21 fish, only one of which was age 6. Our study reported lower overall agreement between whole otoliths and scales (76%), but we examined fish over a much wider age range (ages 0 to 10) than previously reported.

Comparative evaluation of opercular bones

Our comparative studies have found opercular bones to be inferior to both sectioned and whole otoliths in summer flounder, and even to scales. Opercular bones had the lowest confidence scores, the highest reading times, only 46% within reader agreement, and they often exhibited unclear transitions from translucent to opaque zones, particularly at early ages. For these reasons, we feel that opercular bones should not be used for aging summer flounder.

These findings are new for summer flounder, because no previous studies have used opercular bones to age this species. We were disappointed at the poor performance of opercular bones because they have been reported useful in many other species, including perch (LeCren, 1947), carp (McConnell, 1952), yellow perch (Bardach, 1955), northern pike (Frost and Kipling, 1957), tautog (Cooper, 1967; Hostetter and Munroe, 1993), and goldeye (Donald et al., 1992). Many of these studies show photographs of opercular bones with clear, easily recognized marks that have been interpreted as being formed annually. These studies, however, generally have not validated age determination in opercular bones; therefore it is unclear whether they give accurate ages in these other species.

Formation of early marks on otoliths and scales

We sometimes observed an early, presumably false, mark prior to the first presumed annual mark on both otoliths and scales of summer flounder. Although we attempted not to count this early mark, it appeared to be the primary cause for disagreements between our readers in aging both otoliths and scales. This problem has not been reported in summer flounder otoliths, although there is evidence of this early false mark on scales (Dery, 1988; Bolz et al., 2000). Indeed, a primary problem cited by Bolz et al. (2000) for differences in interpretation of summer flounder scales was the choice of a first annual mark.

The early, presumably false, mark that sometimes occurred on summer flounder otoliths and scales appears similar to the first mark reported for Atlantic croaker otoliths (Barbieri et al., 1994a) and might be explained by similarities in certain life history traits of these two species. Both species have a protracted spawning season and spawn over a similar time frame in the Chesapeake Bay region: Atlantic croaker from mid-summer to late fall (Wallace, 1940; Haven, 1957; Barbieri et al., 1994b), and summer flounder from early fall to early winter (Smith, 1973; Morse, 1981; Able et al., 1990). Barbieri et al. (1994a) reported the formation of a first mark on Atlantic croaker otoliths in the first spring following hatching, at 5 to 10 months, with two patterns of early mark formation: 1) the first mark close to, but distinct from, the focus in early hatched fish, and 2) the first mark nearly continuous with the focus in late hatched fish. As with Atlantic croaker, we suggest that the first mark on summer flounder otoliths and scales, which we have referred to as an "early, presumably false, mark," might actually be laid down in the first spring following hatching, at 5 to 8 months, with the same two patterns of early mark formation.

Previous summer flounder aging studies interpret the first annual mark to be laid down on scales and otoliths in the second spring following hatching (Smith et al., 1981; Szedlmayer et al., 1992), at 17 to 20 months, one year after the first annual mark is laid down on Atlantic croaker otoliths. Despite this difference, fish from these two species that are hatched at the same time are currently placed in the same year class. It thus appears that the current age determination methods differ between these two species. For example, according to current conventions (Bolz et al., 2000), a summer flounder hatched in October 2000 would be called age 1 on 1 January 2002, at a biological age of 15 months. This age is several months *before* the first presumed annual mark is laid down on the structures in the second spring following hatching (2002), even though an "early" mark might have been laid down in the first spring following hatching (2001). Similarly, an Atlantic croaker hatched in October 2000 would be called age 1 on 1 January 2002 (Barbieri et al., 1994a), at a biological age of 15 months. However, this age is 8 months *after* the first annual mark is laid down on the otolith, which occurs in the first spring following hatching (2001). Therefore, the two species differ in the way the first annual mark is assigned.

To resolve the issue of early mark formation in summer flounder, we suggest that calcified structures of young-of-the-year fish be examined to determine when the early mark is formed, as Barbieri et al. (1994a) did for Atlantic croaker. Barbieri et al.'s (1994a) validated method automatically assigns an early first mark, formed at 5 to 10 months, to all Atlantic croaker otoliths, whether the mark is distinct or not. If the "early, presumably false, mark" in summer flounder is similar to the first annual mark in Atlantic croaker, an early first mark could likewise be assigned to summer flounder otoliths. If this were done, disagreements on the first mark on summer flounder structures would be fewer, and summer flounder and Atlantic croaker would be aged in exactly the same way. That is, both fish would already have a first annual mark on the structure when ages are advanced to 1 on the 1 January arbitrary birthdate.

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