

Evaluation of opercular bones for aging eight species of Chesapeake Bay fishes

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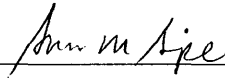
Ann M. Sipe

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This thesis is submitted in partial fulfillment of
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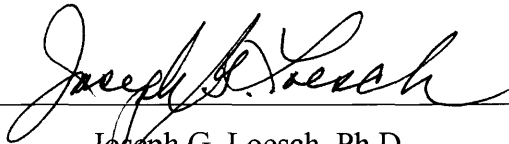


Mark E. Chittenden, Jr., Ph.D.

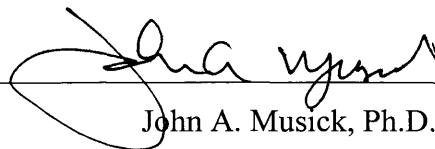
Committee Chair / Advisor



David A. Evans, Ph.D.



Joseph G. Loesch, Ph.D.



John A. Musick, Ph.D.

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ABSTRACT

Opercular bones were evaluated for aging spotted seatrout, weakfish, Atlantic croaker, Spanish mackerel, American shad, and striped bass. Of these species, only striped bass opercular bones showed marks that could be interpreted as annual, although those marks were often inconsistent and difficult to interpret. Striped bass opercular bones showed only 63% agreement in repeated readings and only 47% agreement with sectioned otoliths. In contrast, marks on striped bass sectioned otoliths, previously validated by Secor et al. (1995), were clear and easy to interpret, showing 100% agreement in repeated readings. Opercular bones should therefore not be used to age striped bass unless no other structures are available.

Summer flounder scales, opercular bones, and left and right whole and sectioned otoliths were compared for ages 0 to 10 to identify the best aging method. All structures showed marks that were interpreted as annual, however structures differed greatly in mark clarity. Sectioned otoliths, especially right ones, were the best structure. Right sectioned otoliths consistently showed the clearest marks and had the highest confidence scores and the highest agreement within and between readers. Left sectioned otoliths took twice as long to prepare and were more difficult to interpret than right sectioned otoliths. Whole otoliths, the second best structure, were adequate to age 4 or 5, after which sectioning greatly improved mark clarity. Scales were inferior to whole and sectioned otoliths, tending to overage at younger ages and underage at older ages compared to otoliths. Opercular bones were undesirable for aging summer flounder. They were often difficult to interpret and had the lowest confidence scores and only 46% within reader agreement.

Bluefish scales, whole and sectioned otoliths, dorsal spines, opercular bones, and vertebrae were compared for ages 1 to 14 to identify the best aging method. Opercular bones and vertebral sections were unusable for aging bluefish because they were spongy and pitted and showed no marks. The remaining structures showed marks that were interpreted as annual, however structures differed greatly in mark clarity. Sectioned otoliths were the best structure, consistently showing the clearest marks, the highest confidence scores, and the highest within and between reader agreement for both young and old ages. Whole otoliths were the second best structure, with the second highest within reader agreement and 95% agreement with sectioned otoliths to age 4. Past age 4, whole otoliths were increasingly opaque and sectioning greatly improved mark clarity. Scales were inferior to sectioned and whole otoliths, especially past age 4, when confidence scores for scales averaged only 1.9, within reader agreement was only 33%, and agreement with sectioned otoliths was only 26%. Older fish had problems with thickening of the scale center and crowding of marks at the scale edge. Dorsal spine sections were undesirable for aging bluefish. They were often unclear and inconsistent, having the lowest within and between reader agreement and only 52% agreement with sectioned otoliths. In addition, growth of the vascular core may erode early marks on the dorsal spine sections of larger fish.

Evaluation of opercular bones for aging eight species of Chesapeake Bay fishes

General Introduction

Accurate and precise age determination is fundamental to the proper management of a fishery, as incorrect aging can lead to faulty estimates of yield model parameters and inappropriate management decisions (Beamish and Chilton, 1982; Beamish and McFarlane, 1983). For example, Lowerre-Barbieri et al. (1994) reported that inaccurate aging of weakfish may have led to incorrect estimates of growth and mortality rates, causing management to underestimate that species vulnerability to fishing.

Counting growth rings on calcified structures is the most frequently used method for aging fish because it gives direct information on age (Devries and Frie, 1996). Scales and otoliths are the most widely used aging structures. Less commonly used structures include opercular bones (LeCren, 1947; Hostetter and Munroe, 1993), fin ray and fin spine sections (Lai et al., 1987; Hill et al., 1989), and vertebral centra (Johnson, 1979).

Since calcified structures can have different properties in different species, several authors have recommended that a comparative study of structures be done whenever an aging study on a new species or population is begun (Chilton and Beamish, 1982; Beamish and McFarlane, 1983; Casselman, 1983). Such studies often prove otoliths, especially sectioned otoliths, to be superior to other structures (see Beamish and Chilton, 1982; Lowerre-Barbieri et al., 1994), because they typically continue to form distinct marks at older ages. However, some species have small, difficult to locate, easily broken otoliths, which require considerably more processing time and may have difficult to

interpret marks (Devries and Frie, 1996; Gaichas, 1997). Although scales have long been used to age, they tend to be more difficult to interpret than otoliths, particularly in older fish. Scales also tend to underage past maturity, making them unreliable if one method is needed over broad age ranges (Casselman, 1983; Beamish and McFarlane, 1983).

Opercular bones appear to have desirable aging properties (LeCren, 1947) and may be a better structure in species with inaccurate scales and/or difficult to age otoliths. Opercular bones are easier and cheaper to process than sectioned otoliths, especially in species with small, fragile otoliths, and several studies have shown opercular bones to be better than scales (Donald et al., 1992; Soupir et al., 1997; Vilizzi and Walker, 1999). In addition, opercular bones are externally located and easier to remove than otoliths, a property which might enable recreational fishermen to provide aging materials from the large, old specimens that scientists may not be able to readily find.

Despite their seemingly desirable properties, opercular bones have not been evaluated in most Chesapeake Bay fishes. This thesis presents an evaluation of opercular bones for aging eight species of Chesapeake Bay fishes. Chapter one evaluates opercular bones in spotted seatrout (*Cynoscion nebulosus*), weakfish (*C. regalis*), Atlantic croaker (*Micropogonias undulatus*), Spanish mackerel (*Scomberomorus maculatus*), American shad (*Alosa sapidissima*) and striped bass (*Morone saxatilis*). Chapters two and three evaluate opercular bones in summer flounder (*Paralichthys dentatus*) and bluefish (*Pomatomus saltatrix*), respectively, and are separated from Chapter one because they also include extensive comparative evaluations of other calcified structures, something not previously done for these two species.

Chapter 1

Evaluation of opercular bones for aging six species of Chesapeake Bay fishes

Introduction

Opercular bones have frequently been used to age fish (LeCren, 1947; Bardach, 1955; McConnell, 1952; Frost and Kipling, 1957; Babaluk and Campbell, 1987; Donald et al., 1992; Hostetter and Munroe, 1993), often proving superior to other calcified structures, especially scales (Donald et al., 1992; Hostetter and Munroe, 1993; Soupir et al., 1997; Vilizzi and Walker, 1999). Much of the literature on opercular bones is accompanied by pictures that show clear, easily recognized marks that have been interpreted, and in some cases validated, as annual (for examples, LeCren, 1947; Frost and Kipling, 1957; Babaluk and Campbell, 1987; Hostetter and Munroe, 1993). Opercular bones also have the reported advantages that they are quickly and easily removed, inexpensive to prepare and that their marks become more distinct after the bone has been cleaned and dried (LeCren, 1947; Frost and Kipling, 1957).

Despite their seemingly desirable properties, opercular bones have largely been ignored for aging Chesapeake Bay fishes. The objective of this chapter is therefore to evaluate the opercular bone as a tool for aging six species of important Chesapeake Bay fishes: spotted seatrout (*Cynoscion nebulosus*), weakfish (*C. regalis*), Atlantic croaker (*Micropogonias undulatus*), Spanish mackerel (*Scomberomorus maculatus*), American shad (*Alosa sapidissima*), and striped bass (*Morone saxatilis*). These species were chosen, in part, because most of them already have validated sectioned otolith methods for age determination: spotted seatrout (Ihde, 2000, ages 1 to 5), weakfish (Lowerre-

Barbieri et al., 1994, ages 1 to 5), Atlantic croaker (Barbieri et al., 1994a, ages 1 to 7), Spanish mackerel (Gaichas, 1997, ages 1, 3, and 4), and striped bass (Secor et al., 1995, ages 3 to 7). Thus, by comparing opercular bones to validated otoliths, it is possible to determine if opercular bones give accurate ages in these species. Good agreement between opercular bone and validated sectioned otolith ages would imply a strong measure of validation for opercular bones (Bagenal and Tesch, 1978), which could then be used as a supplemental calcified structure for age determination.

Most of these species (spotted seatrout, weakfish, Atlantic croaker and striped bass) are easily and confidently aged because they have relatively large, easy to section otoliths with very clear marks. However, two of these species, American shad, which has no validated otolith method, and Spanish mackerel, have small, fragile otoliths that are difficult to locate, prepare and interpret, leading to low confidence in the recognition of marks and low precision in repeated readings (Aschenbach, 1997; Gaichas, 1997). In these two species, opercular bones might be a better structure and could potentially be used to strengthen current aging methods and provide alternative means of estimating population dynamics parameters.

An additional benefit of opercular bones is that they may offer the opportunity to more fully use recreational fisheries in data collection. This is important because it is often difficult for scientists to obtain old, large fish specimens with standard sampling sources, and such fish can provide important population dynamics information. Otoliths may be difficult for a nonscientist to find and remove because of their small size and internal location. Opercular bones, however, are large, externally located and easily removed, so that recreational fishermen could potentially provide opercular bones from

the unusually old, large fish that they occasionally catch.

This chapter evaluates opercular bones for aging spotted seatrout, weakfish, Atlantic croaker, Spanish mackerel, American shad and striped bass in the Chesapeake Bay region. Opercular bones were evaluated in terms of the presence of annual marks, the clarity of annual marks, and agreement with sectioned otoliths from the same fish.

Methods

For each species, fish were collected over a broad size range in the Chesapeake Bay region either from commercial fisheries or through collection programs at the Virginia Institute of Marine Science (VIMS). When feasible, collections were made far from the time of annual mark formation to minimize difficulties interpreting marks on the structure edges. Fish were processed for total length (TL) or fork length (FL), total weight (TW), and sex. Both saggital otoliths were removed from each fish and stored dry in tissue culture cell wells, and both opercular bones were removed following LeCren (1947) and frozen until preparation for aging.

Length ranges and sample sizes (n) of fish collections were as follows: spotted seatrout (41 to 816 mm TL, $n = 222$), weakfish (52 to 770 mm TL, $n = 206$), Atlantic croaker (173 to 537 mm TL, $n = 230$), Spanish mackerel (80 to 534 mm FL, $n = 107$), American shad (287 to 524 mm FL, $n = 288$), and striped bass (210 to 1083 mm TL, $n = 175$). Completed collections for each species were stratified into appropriate length categories, and opercular bones from fish in each stratum were chosen to determine if the opercular bones showed any marks that could be interpreted as annual. In species for which opercular bones showed presumed annual marks, a random sample of equal size was chosen from each stratum, and opercular bones were further evaluated for clarity of marks and for agreement in age between opercular bones and otoliths from the same fish.

Opercular bones and otoliths from the same fish were aged independently in a

randomly selected order with no knowledge of the fish size or collection date. At least one week separated the first and second readings of a structure. Ages were assigned based on presumed annual mark counts, and ages that disagreed in the first two readings were reread independently to reach a consensus. Reader comments were evaluated to determine the cause of within and between structure disagreements.

The clarity of marks on opercular bones compared to otoliths was evaluated using simple percent agreement in repeated age readings, readings times, and confidence scores. Confidence scores were assigned on a scale of 1 (low) to 5, and between structure differences in confidence scores were tested at $\alpha = 0.05$ using the normal approximation to the Mann-Whitney test for ordinal data (Zar, 1996).

Agreement in age between opercular bones and otoliths from the same fish was evaluated using simple percent agreement between structures and simple linear regression procedures. For the regressions, opercular bone ages were regressed on otolith ages, and the slope of the regression line was tested to see if it differed significantly from one. A slope of one implies $y = x$ and that the two structures give the same age.

Opercular bones were prepared following LeCren (1947). Briefly, they were soaked in cold tap water for several min to thaw and loosen the skin, then soaked for 1 min in simmering water, after which the skin was removed and the bone was rinsed in water and air dried. Opercular bones were examined with reflected light on a dark background, and presumed annual marks were defined as sharp transitions from narrow translucent zones to wide opaque zones that were continuous from the anterior to the posterior margin of the bone (Hostetter and Munroe, 1993). Translucent zones appeared dark and opaque zones appeared white using reflected light.

Sectioned otoliths were used for comparison with opercular bones in all species except American shad and Spanish mackerel. Either the right or left otolith was mounted sulcal groove down onto cardboard using crystal bond adhesive, and a transverse section was taken through the focus with a variable speed Beuhler Isomet saw. Sections were mounted on top of crystal bond on glass slides, ground using 800 grit PSA sandpaper, and polished with a polishing cloth and 0.3 micron alumina. Sections were viewed at 240X magnification with transmitted light, and annual marks were counted along the sulcal groove as thin opaque bands, which appeared dark with transmitted light.

Whole otoliths of American shad and Spanish mackerel were examined in water on a dark background using reflected light at 240X magnification. Thin opaque bands, which appeared white under reflected light, were counted as annual from the focus to the posterior margin of the otolith.

Results

Opercular bones were not usable for aging spotted seatrout, weakfish, Atlantic croaker and Spanish mackerel. In all but the smallest fish, opercular bones for these four species were consistently yellowed, spongy and pitted (Fig. 1A to 1D), and no marks that could be interpreted as annual were visible on any part of the structure. Corresponding otoliths, in contrast, showed marks that were clear and easy to interpret. Because opercular bones showed no marks, they were not further evaluated in these species.

Opercular bones were also unsuitable for aging American shad, although initial evaluations indicated that they might be useful. American shad opercular bones exhibited concentric opaque bands, and the opercular bones were not yellowed, spongy or pitted (Fig. 2A). However, further examination showed no mark patterns that could be interpreted as annual, even when opercular bones were examined concurrently with whole otoliths from the same fish (Fig. 2B). Because presumed annual mark patterns could not be identified, opercular bones were not further evaluated in American shad.

Initial evaluations of striped bass opercular bones indicated that they might be useful for age determination. Striped bass opercular bones showed marks that could be interpreted as annual in both small and large fish, and they showed few problems with pitting, sponginess, or yellowing of the structure. However, the quality of striped bass opercular bones was variable. Although presumed annual marks were fairly clear on opercular bones of some fish (Fig. 3A), they were inconsistent and difficult to interpret

Figure 1. Examples of unusable opercular bones viewed with reflected light on a black background. No marks are visible on any of the structures. AA = articular apex. Bars = 10 mm. Fish ages noted below are from sectioned (A, B, C) or whole (D) otoliths.

A) Left opercular bone from 595 mm TL, age 3 spotted seatrout collected June 1998.

B) Right opercular bone from 550 mm TL, age 5 weakfish collected September 1998.

C) Right opercular bone from 537 mm TL, age 8 Atlantic croaker collected August 1999.

D) Right opercular bone from 452 mm FL, age 3 Spanish mackerel collected July 1998.

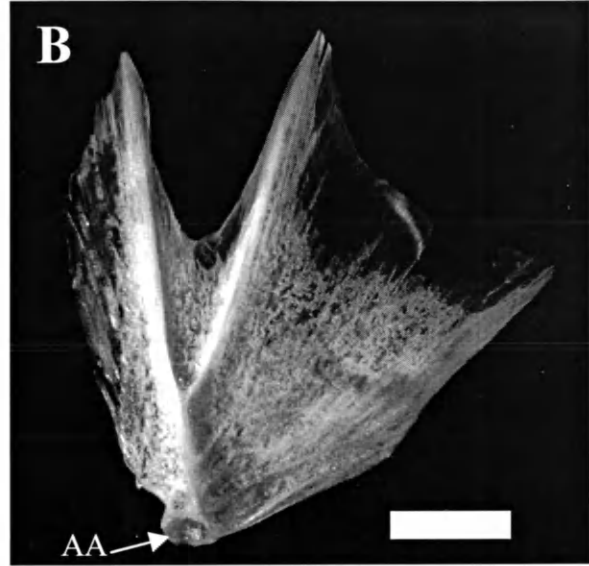
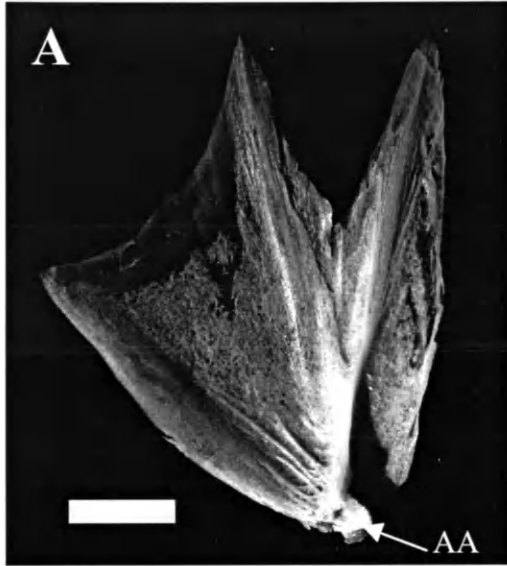


Figure 2. Right opercular bone (A) and right whole otolith (B) from a 524 mm FL, presumed age 10 American shad collected May 1999. Both structures are viewed with reflected light on a dark background. No presumed annual mark patterns are identifiable on the opercular bone. Dots indicate presumed annual marks on the whole otolith. AA = articular apex.

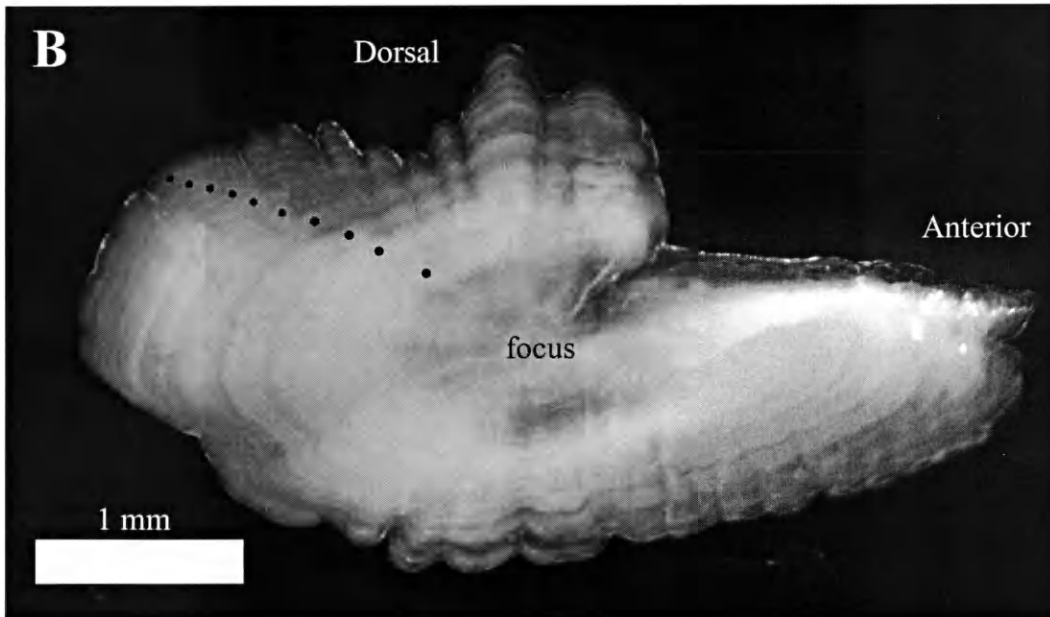
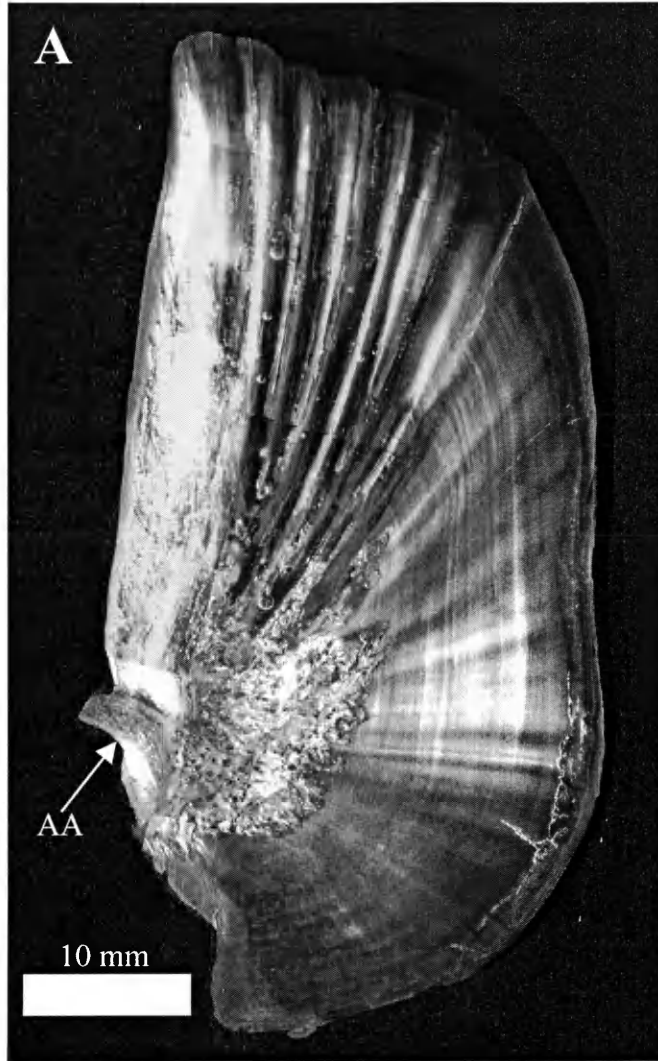
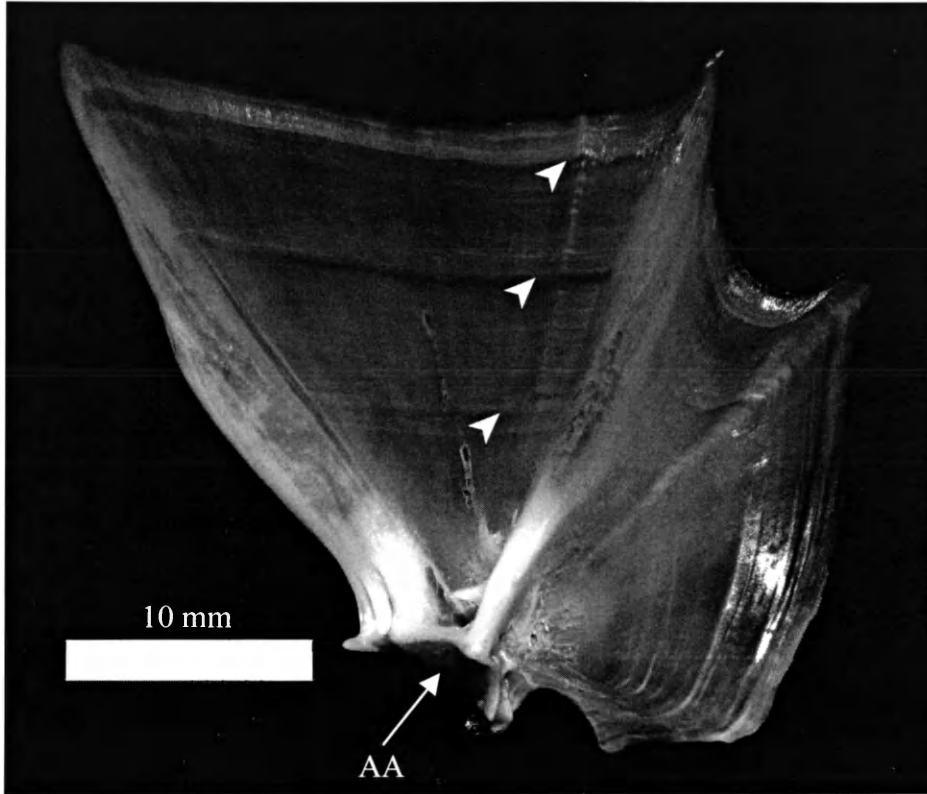
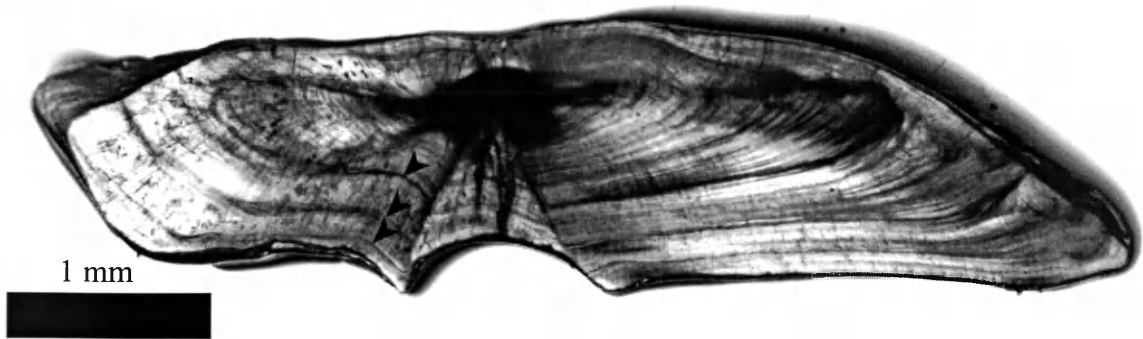


Figure 3. Left opercular bone (A) and sectioned otolith (B) from a 427 mm TL striped bass collected October 1998 and aged 3 using both structures. Arrows indicate individual marks counted. Opercular bone is viewed with reflected light on a black background. Transverse otolith section is viewed in transmitted light. AA = articular apex.

A



B



on opercular bones of other fish, and age readings were often highly subjective. Striped bass sectioned otoliths (Fig. 3B), in contrast, were consistently clear and easy to interpret for fish of all ages. Figure 4 shows a particularly difficult to interpret opercular bone. Marks are narrow and irregularly spaced on this structure, and it appears that one mark may consist of several opaque bands. This opercular bone disagreed in initial age readings and was assigned a confidence score of 1 in all readings. At least 10 marks can be counted on this structure, and the low confidence score indicates that the reader assigned age with great difficulty. In contrast, the sectioned otolith from this fish received a confidence score of 5 and was aged as 7 in all readings.

Mark clarity on striped bass opercular bones was generally inferior to sectioned otoliths. Agreement in repeated age readings was 100% for sectioned otoliths, much higher than the 63% agreement for opercular bones. In addition, the average confidence score of 4.7 for sectioned otoliths was significantly higher than the average of 2.7 for opercular bones ($P < 0.001$). Finally, average reading times were generally higher for opercular bones (0.47 min) than for sectioned otoliths (0.35 min). The lower agreement, lower average confidence scores, and higher average reading times all indicate that marks on opercular bones were more difficult to interpret than marks on sectioned otoliths.

Striped bass opercular bones and sectioned otoliths from the same fish often did not give the same age. Over the age range 0 to 14, I found only 47% agreement between these structures, and the null hypothesis that the slope of the regression line equals one was rejected ($P < 0.001$, Fig. 5). Opercular bones and sectioned otoliths rarely disagreed by more than ± 2 years, showing 88% agreement within ± 1 year and 99% agreement within ± 2 years. Disagreements between opercular bones and sectioned otoliths were

Figure 4. A difficult to interpret left opercular bone from a 546 mm TL striped bass collected October 1998. This fish was aged as 7 with a confidence score of 5 using sectioned otoliths. At least 10 marks can be counted on the opercular bone, and it was only aged with a confidence score of 1 in all readings.

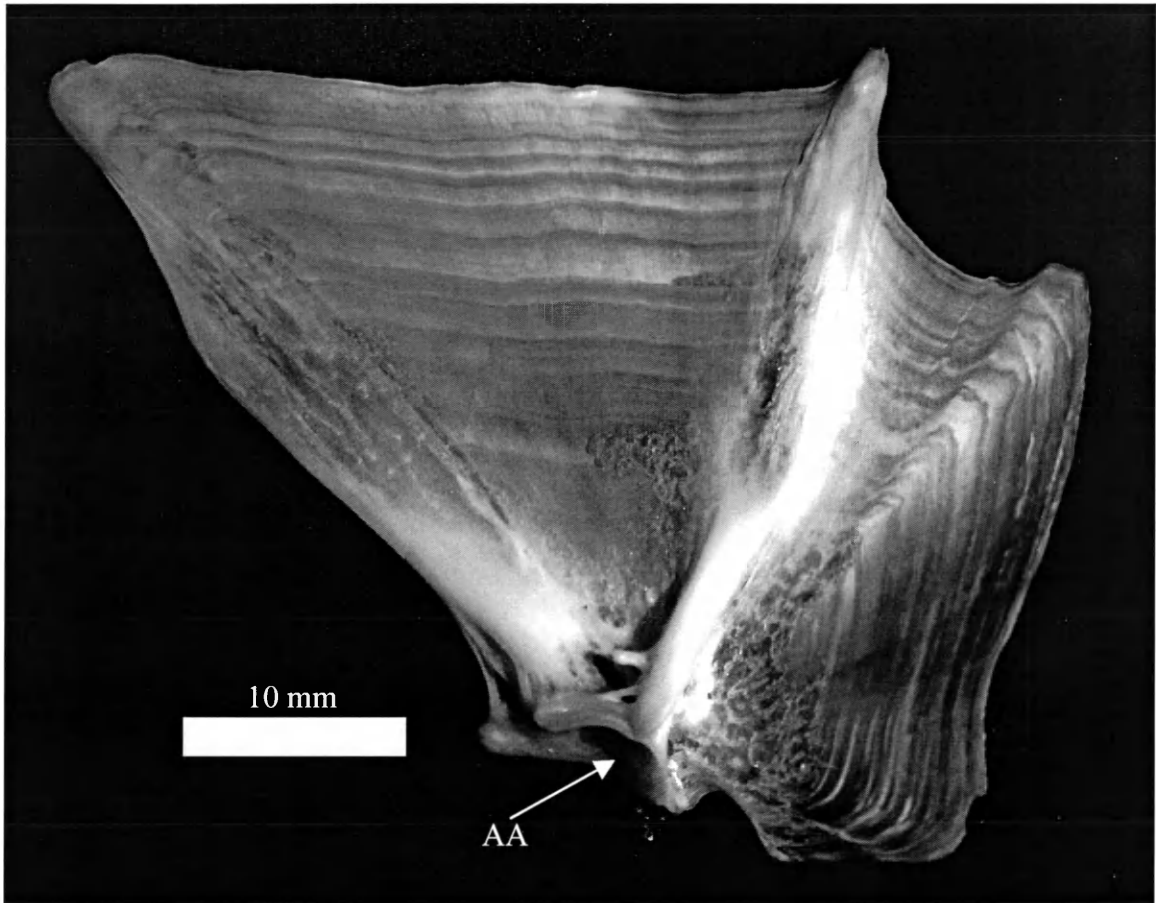
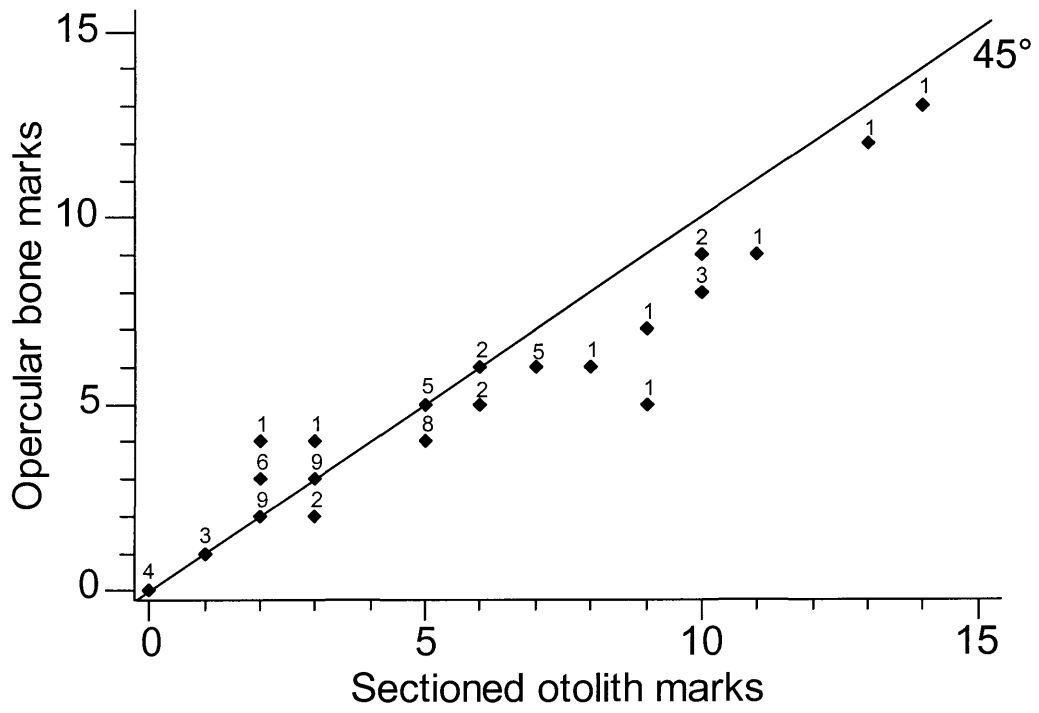


Figure 5. Comparisons of presumed annual mark counts on opercular bones with counts on sectioned otoliths in striped bass. The 45° diagonal line represents 1:1 agreement. The number of fish is indicated at each symbol.



systematic, however, with opercular bones aging higher than sectioned otoliths in nearly all of the disagreements in fish less than age 4 and sectioned otoliths aging higher than opercular bones in all of the disagreements past age 4 (Fig. 5).

These systematic aging differences appear to be related to differences in the thickness of the opercular bone at younger and older ages. At younger ages, when the opercular bone is thinner, it was often difficult to determine what constituted an annual mark because a single mark often appeared to consist of several narrow opaque bands. Hence, opercular bones tended to overestimate age in smaller fish. In larger fish, thickening of the bone caused the narrow opaque bands to appear to merge into one mark, which made the marks more distinct. However, the tendency of opercular bones to be thicker and more opaque in larger fish also caused the early marks to be obscured such that opercular bones often underestimated age in older fish.

Discussion

Opercular bones were unusable for aging five of the six species evaluated in my studies. In spotted seatrout, weakfish, Atlantic croaker and Spanish mackerel, opercular bones were yellowed, pitted and spongy and showed no annual marks. In American shad, opercular bones appeared to have concentric marks, but no annual patterns could be identified on them. These findings are new because no published studies have attempted to use opercular bones for aging these five species. These findings agree, however, with a similar study in bluefish (Chapter 3), where I found opercular bones to be yellowed, pitted, and unusable for aging that species.

Striped bass offered the most promise of the opercular bones studied, as many showed easily recognizable and interpretable marks in initial evaluations. However, many striped bass opercular bones had poor mark clarity, and further evaluation showed them to be inferior to sectioned otoliths, which were previously validated by Secor et al. (1995). Even when opercular bones in striped bass appeared to have good mark clarity, they often provided inaccurate and imprecise ages compared to sectioned otoliths. My observations of mark clarity on striped bass opercular bones agree with previous studies, which found marks to be both quite clear (Scofield, 1931) and indistinct (Merriman, 1941). However, neither of these studies rigorously compared striped bass opercular bones to other structures. Because of the difficulties interpreting marks on opercular bones, I recommend that they not be used to estimate the age of striped bass unless no

other structures are available.

Many of the problems I observed with striped bass opercular bones have been reported in other species. For examples, difficulties interpreting early marks in young fish have been reported by LeCren (1947), McConnell (1952), Cooper (1967), and Vilizzi and Walker (1999). In addition, McConnell (1952) and Vilizzi and Walker (1999) reported that opercular bones of young fish often contained many small accessory marks or “pseudoannuli,” similar to those I found on opercular bones of young striped bass. Finally, the thickening of the bone that I found to obscure early marks in older fish has also been reported by Frost and Kipling (1957), Sharp and Bernard (1988), Wright and Giles (1991), and Hostetter and Munroe (1993).

Many studies have used opercular bones to age fish (for examples, LeCren, 1947; Bardach, 1955; McConnell, 1952; Frost and Kipling, 1957; Donald et al., 1992; Hostetter and Munroe, 1993), and although these studies often presented photographs showing clear marks on the opercular bones, none of them have validated opercular bones to current standards (Beamish and McFarlane, 1983). In most cases where validation was attempted, little or no data was reported, only the youngest ages were validated (see LeCren, 1947; McConnell, 1952, Frost and Kipling, 1957), and when marginal increment analysis was attempted, only pooled age data was used (Cooper, 1967; Hostetter and Munroe, 1993). Opercular bones have rarely been compared to structures other than scales (LeCren, 1947; McConnell, 1952; Frost and Kipling, 1957), and unfortunately, scales have been found to underage at older ages in many species (Chapters 2 and 3; Beamish and McFarlane, 1983; Lowerre-Barbieri et al., 1994; Secor et al., 1995). Indeed, more recent studies that compared opercular bones to both scales and otoliths

have also found scales to underage (Sharp and Bernard, 1988; Wright and Giles, 1991; Soupir et al., 1997). Given the absence of strong validation, the general lack of comparison with structures other than scales, and the difficulties often reported with using opercular bones, I suggest that opercular bone aging be approached with caution unless rigorous validation and between structure comparisons are made.

Chapter 2

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

Introduction

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, 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 summer flounder age determination. 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), which was 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 (Shepherd, 1980), which 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 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 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 age determination using whole otoliths and scales. Previous studies have never evaluated sectioned otoliths in summer flounder, even though sectioned otoliths have often proven superior in other species, especially at older ages when scales and other structures can underage (Beamish and McFarlane, 1983). Further study is especially needed since 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 this chapter was therefore to evaluate and compare whole otoliths, sectioned otoliths, scales, and opercular bones for aging summer flounder. I 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, I discuss the formation of early, presumably false, marks on summer flounder otoliths and scales that resulted in age interpretation difficulties.

Methods

Sample Collection:

To minimize difficulties interpreting marks on the edge of the structures, summer flounder collections 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 following 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 80.8 to 7304.6 g TW and in age from 0 to 10 years (sectioned otolith age).

Preparation of Calcified Structures for Age Determination:

Whole otoliths were examined in water on a dark background using reflected light at 120 to 240X magnification. Thin opaque bands, which appeared white under reflected light, were presumed to represent annual marks (see Fig. 6A). 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. Using 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 using 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 using transmitted light and bright field at 240X magnification. Thin opaque bands, which appeared dark with transmitted light, were presumed to represent annual marks (see Fig. 6B) and were counted along the ventral

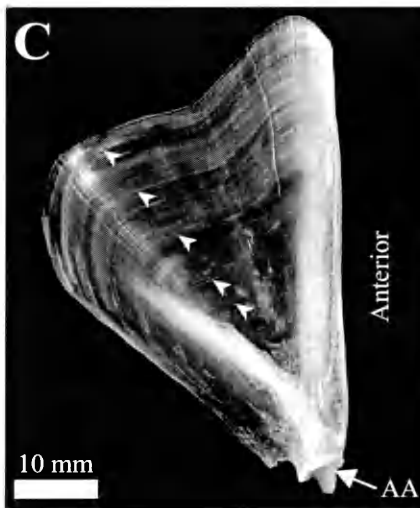
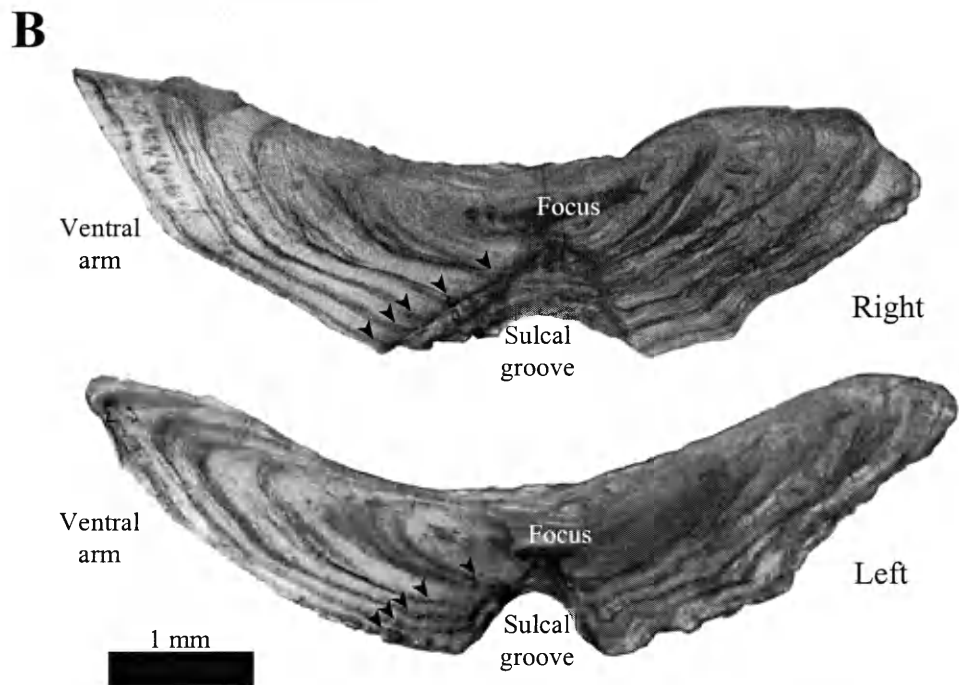
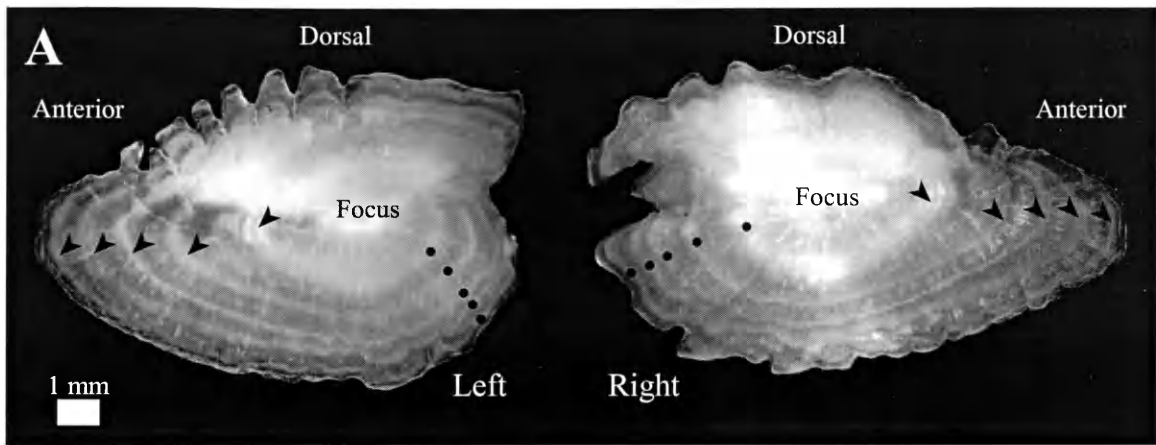
Figure 6. Marks on calcified structures from a 5 year old (sectioned otolith age) female summer flounder, TL = 687 mm, collected in mid-January. Arrows indicate marks.

A) Whole otoliths, viewed in 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.

B) Transverse otolith sections, viewed in transmitted light.

C) Opercular bone, viewed in 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.



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 using a compound video microscope with the Biosonics Optimas image analysis system. 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 following LeCren (1947). Briefly, they were soaked in cold tap water for several min to thaw and partially loosen the skin, then soaked for 1 min in simmering water, after which the skin was easily removed using a toothbrush. The opercular bones were then rinsed with cold tap water and air-dried. Opercular bones were examined dry using transmitted light and in water using reflected light on a dark background. Presumed annual marks (see Fig. 6C) 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 transmitted light and dark under reflected light, while 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 using 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 min at 15,000 pounds of pressure and 60° C. Scale impressions were read using a Bell-Howell R753 microfiche reader at 20x and 32x. Presumed annual marks were identified using standard scale reading criteria as described in Smith et al. (1981), Dery (1988), and Almeida et al. (1992). Briefly, readers enumerated marks (see Fig. 6D) that exhibited cutting over in both lateral fields of the scale 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 using a compound video microscope with the Biosonics Optimas image analysis system.

Evaluation of Calcified Structures:

Each structure was examined for age by two readers, twice by reader one and once by reader two. Structures were read in a randomly selected order with no knowledge of the fish size or collection date. Ages were assigned based on 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 further evaluated 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. My

preliminary evaluation indicated otoliths and scales to be superior to opercular bones, so opercular bones were not further evaluated.

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 $\alpha = 0.05$ 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 the structure size-fish size regressions 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 one were reread independently a third time by reader one to reach a consensus for use in between-

structure comparisons. Likewise, right and left otoliths that disagreed in the initial two readings by reader one 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 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, the x-variable was 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:

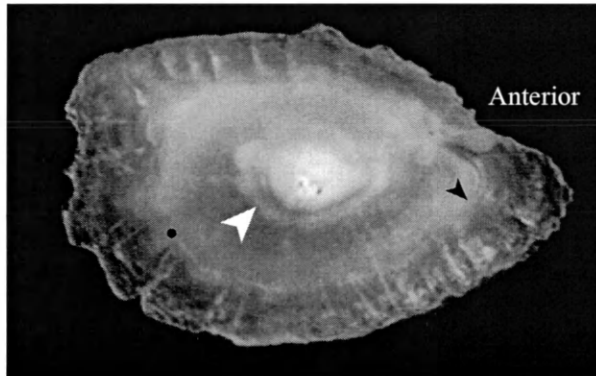
All four calcified structures showed concentric marks that were interpreted as annual (Fig. 6). However, structures differed greatly in presumed annual mark clarity.

Presumed annual marks on both whole and sectioned otoliths (Fig. 6A, 6B) 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. 7). This early mark appeared as a thin opaque band close to, but distinct from, the focus and was found on both young (Fig. 7A) and older (Fig. 7B) fish. An attempt was made not to count this early mark in 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.

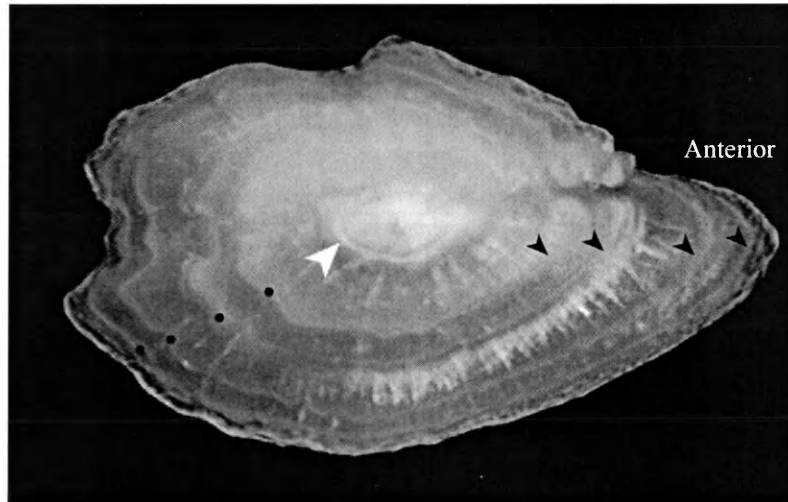
Presumed annual marks on opercular bones (Fig. 6C) were fairly clear in some fish, but they were more often poorly defined, inconsistent, and difficult to follow across the structure, making age interpretation difficult and highly subjective. Opercular bones commonly exhibited unclear transitions from translucent to opaque zones, with the first 1

Figure 7. 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, dots indicate secondary counting path.

A



B



or 2 marks particularly difficult to distinguish, even on young fish. Zone transitions were often easier to interpret towards the structure edge in older fish, although this too varied greatly from fish to fish. The example in Figure 6C is unusually clear and easy to read.

Presumed annual marks on scales (Fig. 6D) were clearer than those on opercular bones, but they still required much subjective interpretation. Figure 6D 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 to get two scales that were adequate to show in Figure 6D. Interpretation of age from scales of older fish was extremely difficult, as marks at the scale edge were often obscured or crowded, particularly in the narrow lateral fields. Finally, a major source of disagreement in scale age determination resulted from an early, presumably false, mark that often occurred prior to the first presumed annual mark (Fig. 6D, asterisk). As this early mark did not appear consistently in all fish or even on several scales from the same fish, an attempt was made not to count it in age readings.

Preparation and Reading times and Confidence in Mark Clarity:

Preparation times were short and reasonable for all structures, at less than 15 min per fish. Whole otoliths took by far the least amount of time, as no preparation was required before reading (Table 1). Sectioned right otoliths and opercular bones required 4 to 6 min to prepare, while scales and sectioned left otoliths took much longer, about 11 and 14 min, respectively. Left sectioned otoliths took so long compared to right

Table 1. Average preparation times (min), reading times (min) \pm standard error (SE), and confidence scores \pm SE for summer flounder calcified structures.

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

sectioned otoliths primarily because they broke much more frequently during sectioning.

Reading times were short and reasonable for all structures, at less than three min per fish. Sectioned right otoliths had by far the shortest reading time, at only 0.27 min per fish (Table 1). Whole otoliths and sectioned left otoliths had the next fastest reading time, at only about 0.4 to 0.6 min 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).

Structure Growth with Fish Growth:

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 ($100r^2$) ranging from 72 to 98%. Values for $100r^2$ were less than 91% only for right and left sectioned otoliths, which were 72 and 85%, respectively.

Table 2. Regression statistics for relationships between structure size and summer flounder total length (TL). Structure abbreviations are defined in the Methods. n = sample size. All regressions were significant at $p < 0.001$.

Structure	Equation	n	$100r^2$
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

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 left whole otoliths, however this could be attributed to the reader learning to use reflected lighting more effectively during the second reading, as 7 of the 9 consensus readings for right otoliths agreed with the second reading. Within reader agreement was lower using 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, I 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 mark clarity and the resulting subjectiveness in scale age readings compared to 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

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

otoliths (paired $t = 17.59$, $df = 73$, $P < 0.0001$; Fig. 6A). However, for sectioned otoliths, the right radial length was significantly longer than the left (paired $t = -11.72$, $df = 43$, $P < 0.0001$; Fig. 6B), because the right otolith is thicker at the focus, where the transverse cross section was taken.

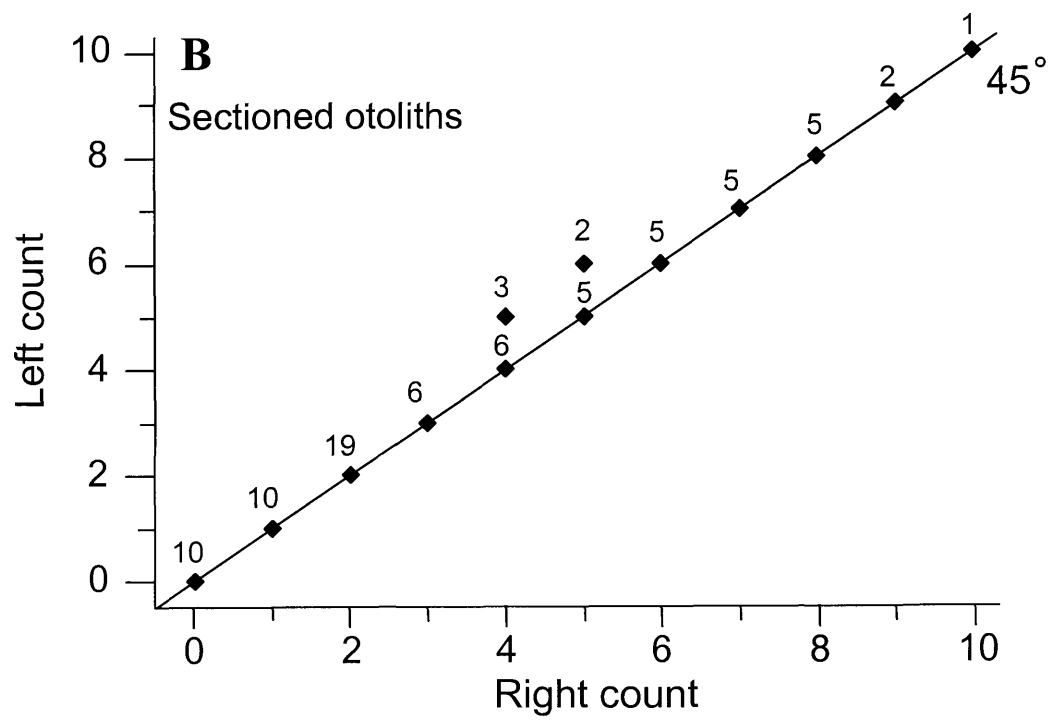
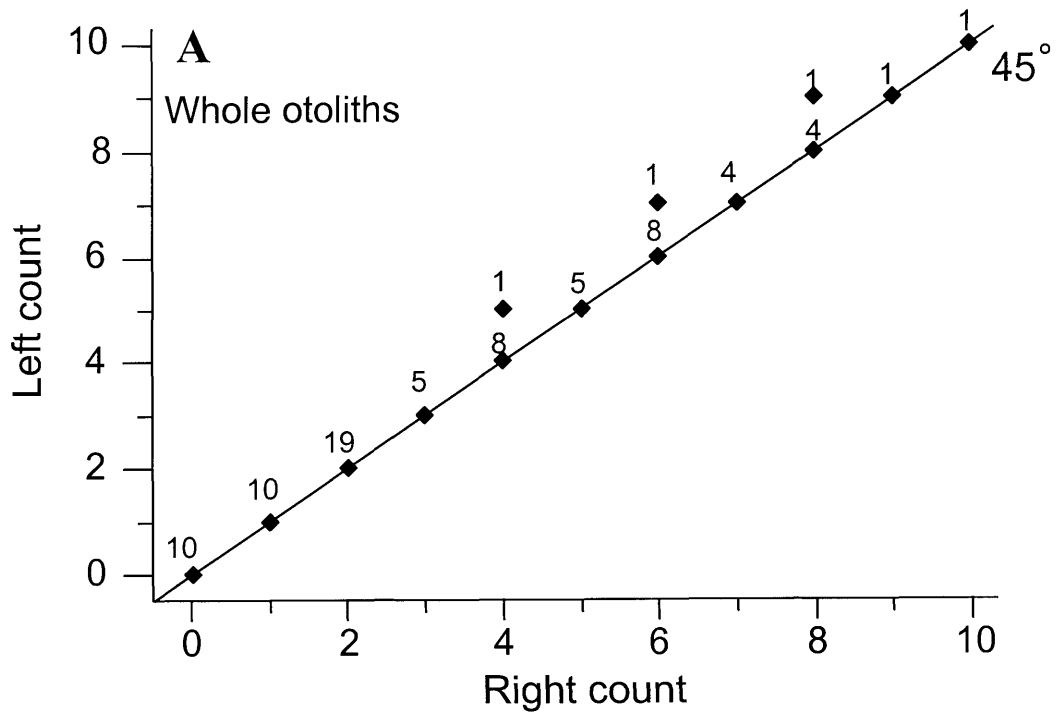
Right and left whole otoliths generally gave the same age readings. Reader one had high age agreement between right and left whole otolith readings (96%), and the null hypothesis that the slope of the line equals one was not rejected ($P = 0.077$, Fig. 8A).

Although right and left whole otoliths generally gave 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 its greater distance between the focus and the posterior margin (Fig. 6A). This greater distance made the marks further apart and more easily distinguished 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. 8B).

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 crowded and less clearly defined (Fig. 6B).

Figure 8. Comparisons of presumed annual mark counts on the left otolith with mark counts on the right otolith for whole otoliths (A) and sectioned otoliths (B) in summer flounder. The 45° diagonal line represents 1:1 agreement. The number of fish is indicated at each symbol.



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. 9A). In addition, the null hypothesis that the slope of the line equals one was not rejected ($P = 0.901$).

Although whole and sectioned otoliths generally gave 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. That 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. 10A), with all confidence scores being 5. Marks were less clear on the whole otolith (Fig. 10B), however, with between 8 and 10 marks counted in different readings, and an average confidence score of only 2.5. In general, sectioning the 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. 9B). In addition, the null hypothesis that the slope of the line equals one was rejected ($P = 0.047$). Scales tended to overage compared to sectioned otoliths in fish age 4 and younger, but underage in fish older than age 4.

Figure 9. 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.

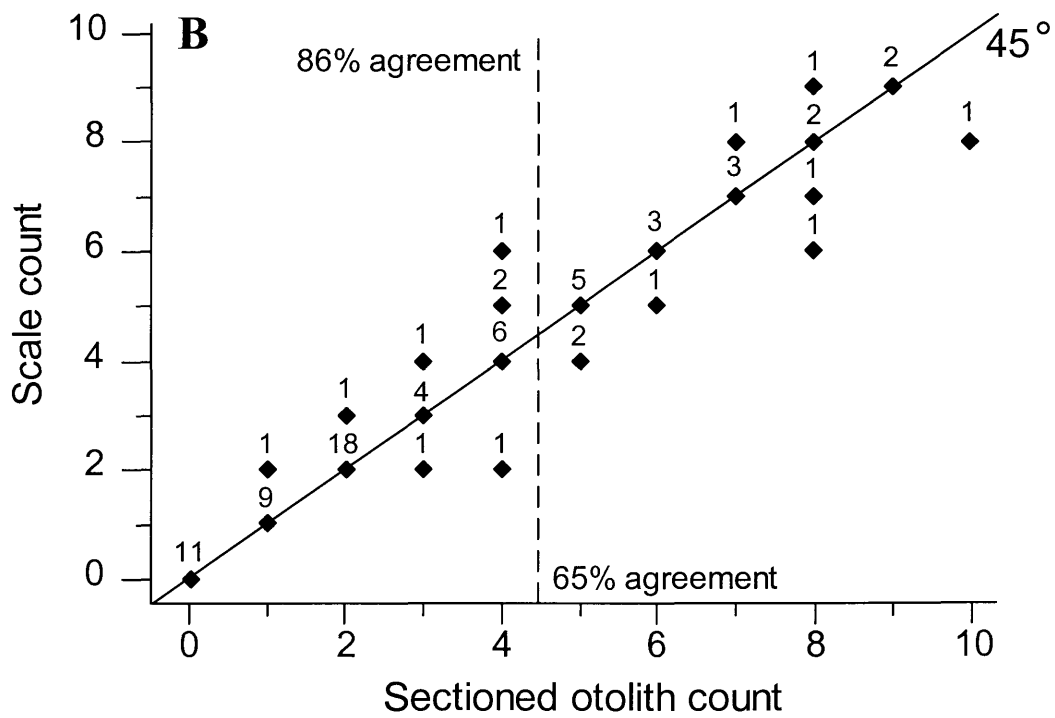
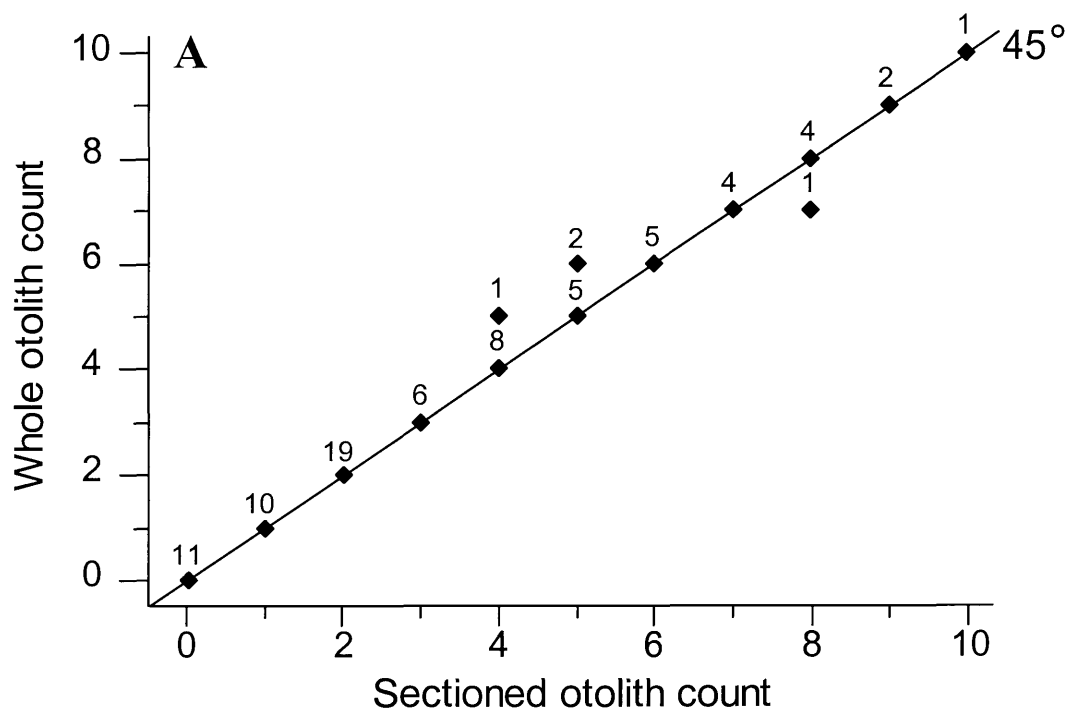
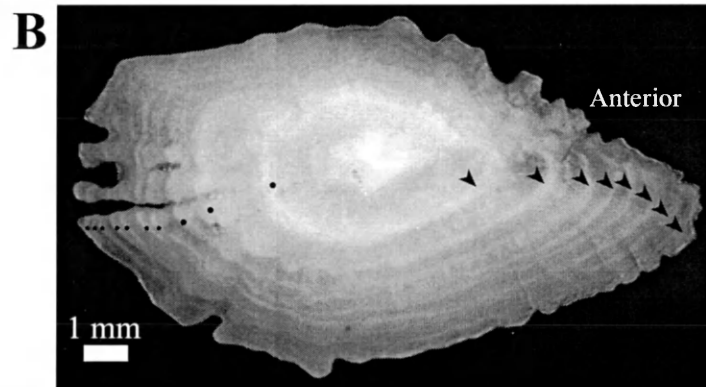


Figure 10. Right sectioned (A) and whole (B) otolith from a female summer flounder, 10 years old (sectioned otolith age) and 758 mm TL, collected in November. Arrows on the sectioned otolith indicate presumed annual marks. On the whole otolith, arrows indicate primary counting path, dots indicate secondary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



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. 11). 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 to whole otoliths in fish age 4 and younger and 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 grew, 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 $100r^2$ values were high, generally 80 to 86%. Values for $100r^2$ 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 $100r^2$ values from 83 to 86%.

Figure 11. 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.

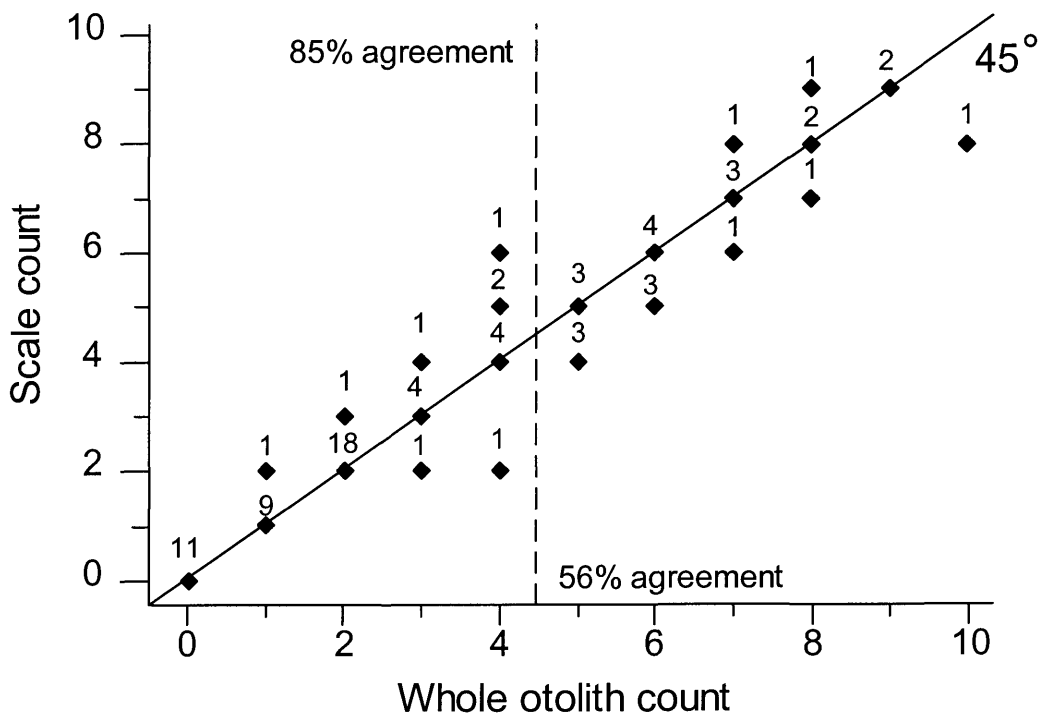


Table 4. Regression statistics for relationships between the number of marks (Marks) and calcified structure size for summer flounder. Structure abbreviations are defined in the Methods. n = sample size. All regressions were significant at $p < 0.001$.

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

Discussion

Comparative evaluation of sectioned otoliths:

I found sectioned otoliths to be the best structure for aging summer flounder over the age range 0 to 10 years. Sectioned otoliths had the lowest 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 in 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 I found high agreement in age between right and left sectioned otoliths, right otoliths were much easier to prepare, and they had a wider counting path, which made it easier to identify the marks, resulting in lower reading times, higher confidence scores, and higher reader agreement.

Although sectioned otoliths were the best structure for determining the age of summer flounder, this study has not proven their accuracy. However, until validation is done, I 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:

I found whole otoliths to be the second best structure for aging summer flounder over the age range of 0 to 10 years. Whole otoliths had no preparation time, the second lowest 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 I feel they are adequate for these younger ages, especially in large-scale production aging where preparation time is important.

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

My 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 my findings, I do not agree with the current preference for using scales rather than whole otoliths in summer flounder. Indeed, I 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) obscuring of the first mark as the fish ages (Powell, 1982), 3) deviation from the generalized pattern of opaque and translucent zone formation in temperate fishes (Smith et al., 1981), and 4) a narrow opaque zone as compared to the translucent zone (Smith et al., 1981). These issues are addressed in turn below.

I rarely observed poor calcification or poor contrast between opaque and translucent zones of whole otoliths. Rather, my procedures gave good contrast between opaque and translucent zones, so that there was high confidence in age readings. In addition, only one otolith of 81 pairs was poorly calcified. This otolith was easily aged upon sectioning, and its pair otolith was not poorly calcified and was aged with high confidence.

I saw little evidence that the first mark becomes obscured at older ages on whole otoliths, as indicated by the high agreement I found 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.

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. I saw no evidence of this reversal. My fish were collected from October through December, so I should have observed opaque edges on the otolith if the timing of mark formation were reversed from other temperate fishes. Instead, I 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), I 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. I disagree with their analysis, however, because many other fishes in my 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 properly be used for age determination if the mark can be proven annual.

Comparative evaluation of scales:

I found scales to be 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. I recommend that scales not be used for aging summer flounder if otoliths, especially sectioned otoliths, are available.

The difficulties I 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 my study, 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 I 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. My study reported lower overall agreement between whole otoliths and scales (76%), but I examined fish over a much wider age range (ages 0 to 10) than previously reported.

Comparative evaluation of opercular bones:

My 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, I recommend that opercular bones 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. The poor performance of opercular bones was surprising, 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 annual. These studies, however, generally have not validated age determination in opercular bones, so it is unclear if they give accurate ages in these other species.

Formation of early marks on otoliths and scales:

Readers sometimes observed an early, presumably false, mark prior to the first presumed annual mark on both otoliths and scales of summer flounder. Although readers attempted not to count this early mark, it appeared to be the primary cause for both otolith and scale aging disagreements between readers. 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 in Atlantic croaker, I suggest that the first mark on summer flounder otoliths and scales, which I 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 one on January 1, 2002, at a biological age of 15 months. This 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 one on January 1, 2002 (Barbieri et al., 1994a), at a biological age of 15 months. However, this is 8 months *after* the first annual mark is laid down on the otolith, which occurs in the first spring following hatching (2001). The two species therefore differ in the way the first annual mark is assigned.

To resolve the issue of early mark formation in summer flounder, I 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 one on the January 1 arbitrary birthdate.

Chapter 3

A comparison of calcified structures for aging bluefish, *Pomatomus saltatrix*

Introduction

The bluefish, *Pomatomus saltatrix*, is a pelagic schooling species distributed throughout the world in temperate and warm-temperate zones, generally in continental shelf waters (Briggs, 1960). In the western Atlantic, bluefish range from Brazil and Argentina to Cape Cod, Massachusetts, occasionally straying northward to Nova Scotia (Bigelow and Schroeder, 1953; Leim and Scott, 1966). In the North Atlantic, bluefish winter off the southeastern United States and migrate north in the spring, generally as far as Massachusetts, reversing this pattern in the fall (Hildebrand and Schroeder 1928, Wilk 1977). The bluefish, a major target of recreational fishermen, is one of the most economically important recreational fishes on the Atlantic coast, with the commercial fishery contributing a relatively minor economic role (Wilk, 1977; MAFMC, 1990).

Despite their economic importance, relatively few age determination studies exist for bluefish. Prior to 1980, workers relied solely on scales for aging this species (Hamer, 1959; Backus, 1962; Lassiter, 1962; Richards, 1976; Wilk, 1977). Several problems have been reported with scale use, however, including a high incidence of regenerated scales, false annuli, and different age readings between scales from the same fish (Backus, 1962; Lassiter, 1962; Richards, 1976). In addition, precision in repeated readings has never been reported for bluefish scales, and it is unknown if bluefish aging is accurate because no studies have presented data to validate the scale method.

More recent work has examined structures other than scales (Barger, 1990;

Chiarella and Conover, 1990), and otoliths have emerged as a viable structure for aging bluefish. Barger (1990) found whole otoliths to have much higher between reader agreement (92%) than scales (67%) in Gulf of Mexico bluefish. However, Barger did not report within reader agreement, between structure agreement, or the age range of the fish he used in his comparison, so it is difficult to evaluate if the structures are equally useful at both young and older ages. Chiarella and Conover (1990) reported 93% agreement in a comparison of scales and sectioned otoliths in 29 bluefish age six and younger. It is questionable whether their agreement would have remained high had they looked at older fish, however, as recent studies on a variety of species have shown that scales often begin to underage compared to sectioned otoliths as fish age increases (see Chapter 2; Beamish and McFarlane, 1983; Lowerre-Barbieri et al., 1994).

Further evaluation of bluefish calcified structures is needed, especially given the economic importance of this species and the reported difficulties in age determination using scales, the primary structure used to date. Although otoliths appear promising for aging bluefish, no studies have rigorously evaluated them over all size classes. The objective of this study was therefore to compare new and previously used calcified structures from a wide size range of bluefish to identify the optimal structure for age determination. Sectioned otoliths, whole otoliths, scales, dorsal spines, opercular bones and vertebrae were examined for aging bluefish. Calcified structures were evaluated in terms of the presence of presumed annual marks, preparation and reading times, confidence scores for age readings, precision in repeated age readings, agreement between different structures of the same fish, structure growth with fish growth, and increases in the number of presumed annual marks with structure size and fish size.

Methods

Sample collection:

Bluefish were collected from commercial fisheries and recreational tournament sources in the Chesapeake Bay region in May and June 1999 ($n = 63$) and September through November 1999 ($n = 43$). Additional unusually large fish were collected in February 2000 near Cape Hatteras ($n = 8$). To minimize difficulties in interpreting marks on the structure edges, I attempted to collect fish far from the time when presumed annual marks form, reported to be May and June on bluefish scales (Wilk, 1977; Terciero and Ross, 1993). However, because of the migratory nature of bluefish, I was unable to acquire fish from all size classes in the same time period. Although Wilk (1977) and Terciero and Ross (1993) presented no data to support the timing of mark formation, my observations that fish collected in May and June were in the process of forming a mark on their structures support their findings.

Fish were processed for fork length (FL), total weight (TW), and sex, and the calcified structures were removed as follows. Both saggital otoliths were removed from each fish, wiped clean and stored dry in tissue culture cell wells. Scales were removed from an area near the tip of the left pectoral fin below the lateral line (Richards, 1976) and stored dry in coin envelopes. Both opercular bones were removed following LeCren (1947), and the spinous dorsal fin and entire vertebral column were removed and frozen until preparation for aging.

Fish collected in 1999 ranged in size from 226 to 839 mm FL. To include as many age groups as possible in the final study sample, the 1999 collection was stratified into 3 length-based categories: 201 to 400 mm, 401 to 600 mm, and 601+ mm FL. A random sample of 20 fish was then chosen from each category, for a total of 60 fish. These 60 fish were then supplemented with the 5 largest fish (810 to 864 mm FL) of those collected in February 2000, bringing the total final study sample to 65 fish. All calcified structures in the final study sample were assigned random numbers before preparation and aging. Bluefish in the final study sample ranged in size from 226 to 864 mm FL and 147.7 to 8418.8 g TW and in age from 1 to 14 years (sectioned otolith age).

Preparation of calcified structures for aging:

Dorsal spines were soaked for 1 min in simmering water, after which adhering tissue was wiped away and the structure was rinsed in tap water and allowed to air dry. A preliminary review of all dorsal spines from two fish (525 mm and 809 mm FL) indicated that a sectioning distance of 4 mm from the base of the fourth or fifth dorsal spine resulted in the most distinct marks and the smallest vascular cores. The fifth dorsal spine was mounted on cardboard using crystal bond adhesive, and a 0.5 mm thick cross section was taken 4 mm from the base of the spine using a variable speed Beuhler Isomet saw. The fourth spine was sectioned if the fifth spine was unavailable or damaged. Sections were mounted on top of crystal bond on glass slides and viewed at 1000X magnification using both transmitted and reflected light. Presumed annual marks were identified as relatively wide opaque bands, which appeared dark in transmitted light and white in reflected light, and were separated by narrow translucent bands.

Scales were initially examined under a dissecting scope to locate 4 or 5 that were unregenerated and symmetrical, thus acceptable for pressing. Acceptable scales were soaked in warm water until flexible, brushed gently with a soft bristle toothbrush to remove debris, and air-dried. They were then taped to an acetate sheet, inserted between two blank acetate sheets, and pressed in a Carver laboratory scale press for 1.5 to 2 min at 10,000 pounds of pressure and 66 to 71° C. Scale impressions were read using a Bell-Howell R753 microfiche reader at 20x and 32x magnification. Presumed annual marks were identified using standard scale reading criteria as described in Bagenal and Tesch (1978). Briefly, readers enumerated marks that exhibited cutting over in both lateral fields of the scale. In young fish, cutting over was accompanied by a clear narrow zone in the anterior portion of the scale, but this was a less reliable criterion in older fish.

Whole otoliths were examined in water on a dark background using reflected light at 120 to 240X magnification. Either the left or right otolith was chosen in a preliminary evaluation based on which one exhibited the clearest marks. Thin opaque bands, which appeared white under reflected light, were presumed to represent annual marks. The entire otolith was examined for marks, and then two counting paths were used for mark enumeration: 1) from the focus to the anterior margin, and 2) from the focus to the posterior margin.

After whole otolith readings were made, the otolith that was read whole was mounted sulcal groove down onto cardboard using 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 top of crystal bond on clear glass slides and were ground using 800 grit PSA sandpaper and polished with a polishing cloth and

0.3 micron alumina. Sections were viewed at 240X magnification using both transmitted and reflected light. Thin opaque bands, which appeared dark with transmitted light and white with reflected light, were presumed to represent annual marks and were counted along the dorsal side of the sulcal groove.

Opercular bones were prepared following LeCren (1947). Briefly, they were soaked in cold tap water for several min to thaw and partially loosen the skin, then soaked for 1 min in simmering water, after which the skin was easily removed using a toothbrush, then air dried.

The tenth vertebra anterior to the hypural plate (Barger, 1990) was removed from the vertebral column and soaked in a 1:1 bleach:water solution for 30 min until tissue could be removed from it. Then it was rinsed with hydrogen peroxide to neutralize the bleach, rinsed with water, and air dried. A preliminary review of vertebrae from two fish (445 and 771 mm FL) indicated that readings of the whole structure were not feasible because the cone surface is too deep. Several methods were attempted to improve mark clarity, including staining (Johnson, 1979; Barger, 1990) and taking both longitudinal and transverse sections (see Cailliet et al., 1983 for description of sectioning plane), although neither of these methods improved readability.

Evaluation of calcified structures:

Each structure that exhibited presumed annual marks was examined for age by two readers. Each reader did one practice reading of each structure to become familiar with that structure. Then reader one did two additional readings, and reader two did one additional reading. Structures were read in a randomly selected order with no knowledge

of the fish size or collection date. Different structures from the same fish were read independently, and at least one week separated the first and second readings of the same structure by a reader. Raw ages were assigned based on presumed annual mark counts.

Reader comments on the structures were used to determine the cause of disagreements within and between readers. After all age readings were finished, and prior to evaluating structures for percent agreement, reader comments were used to adjust presumed ages for fish caught in May and June, during the time of mark formation. For example, if a fish captured in May or June was aged as 1+ with the comment “a lot of plus growth” or “mark may be forming,” then age was advanced to 2. The purpose of this was to exclude disagreements that were due solely to mark formation. Adjusted ages were used in all further analyses.

Structures were evaluated in terms of the following criteria: 1) the presence of presumed annual marks, 2) preparation time, 3) reading time, 4) confidence in the clarity of presumed annual marks, 5) agreement in repeated age readings of the same structure (precision), 6) agreement in age readings between different structures from the same fish, 7) growth of the structure with size of the fish, and 8) increases in the number of presumed annual marks with increases in structure and fish size.

Preparation time, a measure of the processing efficiency of a structure, was evaluated as the time taken to prepare structures for reading after they were collected and stored. Reading times and confidence scores were used to evaluate the clarity of presumed annual marks on a structure. Reading time was measured as the time taken to read a given structure in an individual fish. Confidence scores were assigned by the reader to each reading and were expressed on a scale of 1 (low) to 5 (high). Differences

in confidence scores between structures were tested at $\alpha = 0.05$ using the normal approximation to the Mann-Whitney test for ordinal data (Zar, 1996).

Precision in age determinations for a given structure was evaluated using simple percent agreement in repeated readings within and between readers. Agreement within \pm one year was also calculated to indicate the extent of the disagreements. 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 reader one to reach a consensus. Between reader agreement compared the consensus age of reader one with the first reading of reader two.

Agreement in presumed annual mark counts between different structures of an individual fish was evaluated using simple percent agreement and linear regression procedures. For the regressions, 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 one. A slope of one implies that $y = x$ and that the two structures give the same age. For each regression, the x-variable was the structure judged superior based on confidence scores and within and between reader agreement.

The assumptions that structure growth is directly related to fish growth and that that the number of presumed annual marks on a structure is directly related to structure size and to fish size were evaluated using linear regression (Zar, 1996). To determine if these relationships were significant and increasing, structure sizes (DSSA, ScRL, WOTL, SORL) were regressed on fish FL, and the number of marks on a structure was regressed on structure size and on fish FL. Although some of these relationships showed curvature, they were not tested for curvature using more parameterized models because the

predictive ability of the regressions was not important in these evaluations. Sample sizes varied in these regressions because some structures were fractured and could not be measured. Calcified structure sizes were measured using a calibrated digital imaging system and SPOT RT software version 3.0 (Diagnostic Instruments, Inc., 1999). Dorsal spine sections were measured for total sectional surface area (DSSA) and for vascular core area. The most symmetrical scale was chosen and measured for scale radial length (ScRL) from the center of the focus along the ventral axes 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 core, 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. Whole and sectioned otoliths were not measured if they were fractured along the measuring path.

Results

Comparative appearance of calcified structures:

Initial evaluations indicated opercular bones and vertebrae were not useful in aging, so these structures were eliminated from further comparisons. For fish of all sizes, the opercular bones were consistently yellowed, spongy and pitted with no marks visible anywhere on the structure (Fig. 12A). Whole vertebral cone surfaces showed concentric rings, but no presumed annual mark patterns could be identified, making determination of age highly subjective with low confidence. Sectioning of vertebrae was not a viable solution because the interior of the vertebra was spongy with no marks visible (Fig. 12B).

Dorsal spines, scales, and whole and sectioned otoliths showed concentric marks that could be interpreted as annual (Fig. 13). However, these structures differed greatly in presumed annual mark clarity, with age generally the biggest factor and younger fish being the easiest to interpret for all structures but dorsal spines.

Presumed annual marks on dorsal spines (Fig. 13A) were fairly clear in some fish, but were more often poorly defined, inconsistent, and difficult to follow around the structure in both young and old fish. As such, dorsal spine readings were often highly subjective, resulting in low confidence in the age readings. The first mark was particularly difficult to identify on young fish and was a large source of disagreement between readers and between structures. Figure 14 shows some of the common problems encountered with dorsal spines from large fish, including the existence of a large vascular

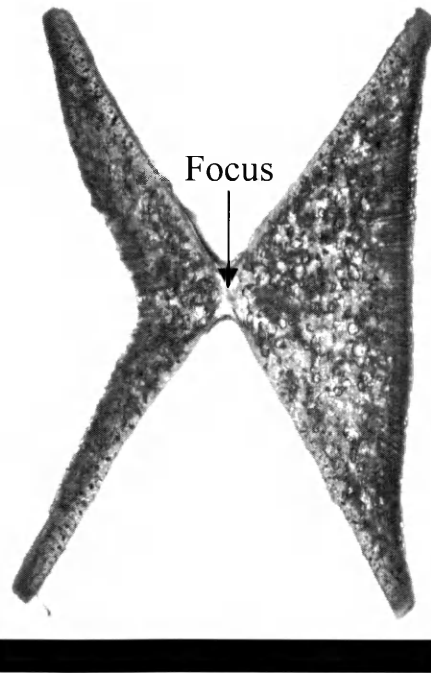
Figure 12. Opercular bone (A) and longitudinal vertebral section (B) taken from a 445 mm FL, 1 year old (sectioned otolith age) female bluefish collected 26 October 1999. No marks are visible on either structure. Bars = 10 mm.

A



Articular
apex

B



Focus

Figure 13. Presumed annual marks on calcified structures taken from a 6 year old (sectioned otolith age) female bluefish, 724 mm FL, collected 22 November 1999. Arrows indicate individual marks counted as described in the Methods. Bars = 1 mm.

A) Dorsal spine section, viewed in transmitted light. Only 5 marks were counted on this structure.

B) Scale impression, viewed in transmitted light. Middle of scale is thickened and pitted, making identification of the first mark difficult.

C) Whole otolith, viewed with reflected light on a black background. Arrows indicate presumed annual marks in the anterior field, dots indicate presumed annual marks in the posterior field.

D) Transverse otolith section, viewed in transmitted light.

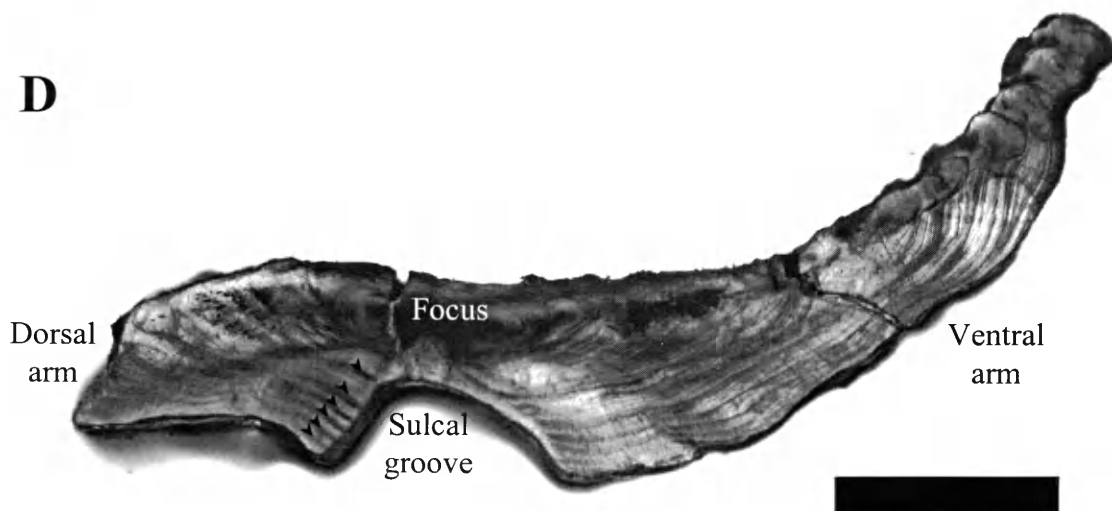
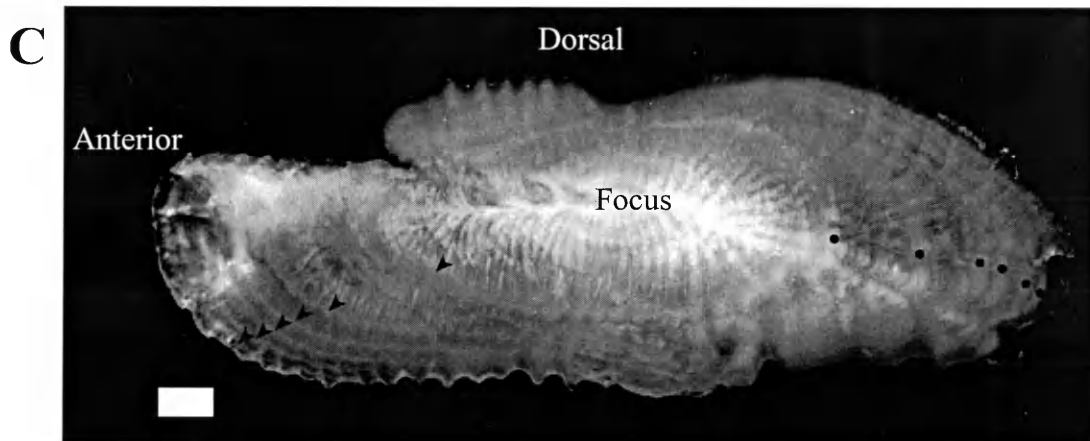
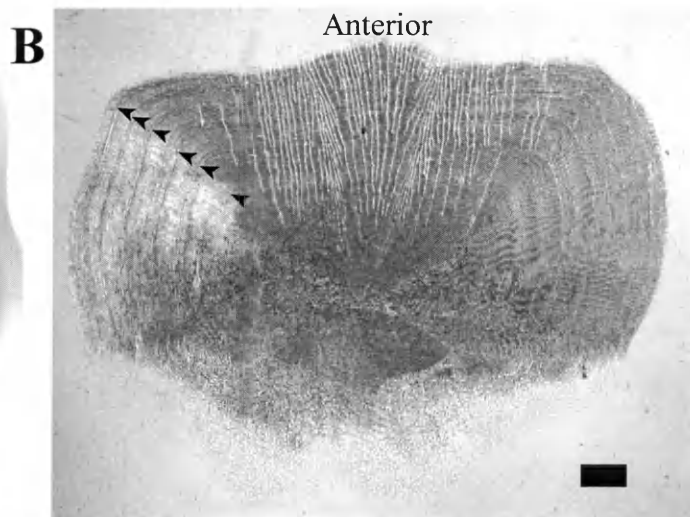
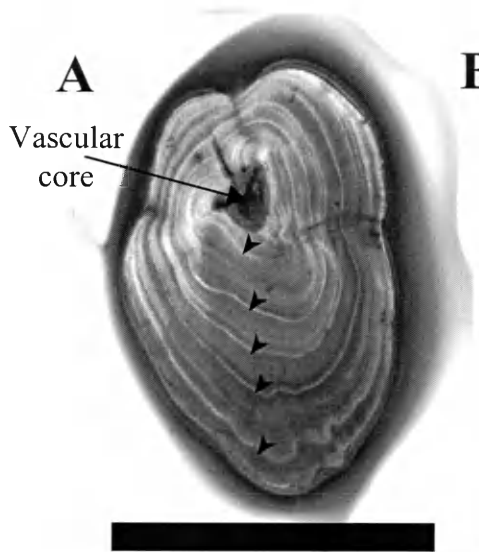
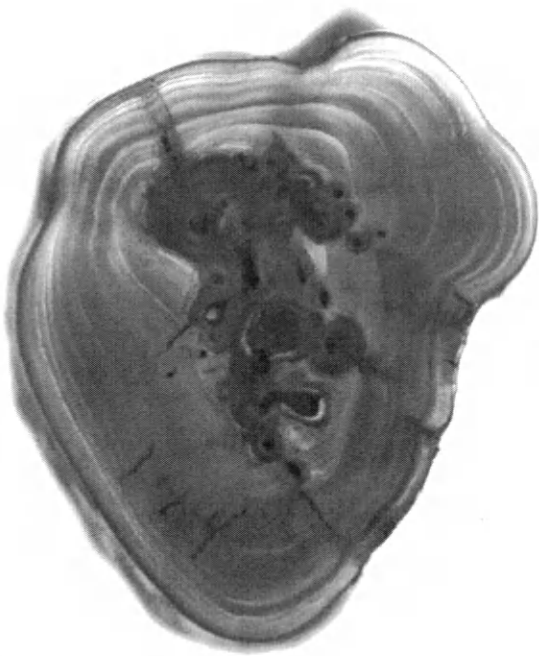


Figure 14. Dorsal spine sections from two bluefish collected 30 November 1999 showing large vascularized cores, narrow or light edge marks, and poor separation between marks. The section on the left is from an age 8 male (sectioned otolith age), 737 mm FL, and the one on the right is from an age 9 female, 790 mm FL. Bar = 1 mm.



core, light and narrow edge marks, and poor separation of marks.

Presumed annual marks on scales (Fig. 13B) were typically clear and easy to read in younger fish, but age determination from scales of older fish was often difficult and more subjective. False marks, indicated by crossing over in only one lateral field, were a common problem with scales and were particularly noticeable in younger fish.

Interpretation of age in older fish was extremely difficult because the center of the scale became thickened and pitted, which tended to obscure early marks. In addition, marks became crowded at the scale edge, so that it was difficult to distinguish one mark from another. Finally, many fish had regenerated, assymetrical, or otherwise damaged scales, making it difficult and time consuming to choose acceptable scales to press. For example, of the 65 fish used in the structure comparison, two had no unregenerated scales, and six had only one or two unregenerated scales. Regenerated scales were more common in larger fish; over half the scales were regenerated in 80% of fish over 500 mm FL, and over 90% of the scales were regenerated in 20% of fish over 500 mm FL.

Presumed annual marks on whole otoliths (Fig. 13C) were fairly clear in younger fish, but they 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, causing marks to be poorly defined and making age determination for these fish difficult and highly subjective. In addition, older fish often exhibited crowded or faint marks at the structure edge.

Presumed annual marks on sectioned otoliths (Fig. 13D) were typically the most consistent and easiest to interpret, especially in younger fish. Disagreements in sectioned otolith ages were more common at older ages and usually reflected a lightening of

presumed annual marks towards the structure edge. This did not occur in all older fish, but it was a common source of disagreement when it did occur. In addition, some sectioned otoliths of older fish showed what appeared to be double marks along the sulcal groove. These occurred in about 5% of my sample and were generally corroborated by double marks on the edge of the dorsal arm of the structure. Sectioned otoliths were difficult to prepare because they tended to fracture during sectioning, but this problem was largely alleviated with experience.

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

Preparation times were reasonable for all structures, at less than 20 min per fish. Whole otoliths required no preparation time before reading (Table 6). Sectioned otoliths, scales and dorsal spines had similar preparation time, averaging 14.3 to 16.7 min per fish. Preparation time for both scales and sectioned otoliths decreased with experience, from an average of 16.6 and 20.3 min, respectively, in the first 20 structures to an average of 12.7 and 13.4 min, respectively, in the last 20 structures. There was no decrease in preparation time for dorsal spines. Due to the high incidence of regeneration, 9.2 min (64%) of the scale preparation time was needed to find acceptable scales for pressing.

Reading times were fairly high for all structures, at close to 2 min per fish. Sectioned and whole otoliths had the shortest average reading times, at 1.7 min per fish (Table 6). Scales and dorsal spines had slightly longer reading times, at 1.8 and 2.0 min per fish, respectively. Long reading times, such as those I found in all bluefish structures, usually indicate difficulty in interpreting marks.

Reader confidence scores were generally lower than desirable, and they varied

Table 6. Average preparation time and reading time (min) \pm standard error (SE) and average confidence score \pm SE overall and by age for bluefish calcified structures.

Structure	Preparation Time	Reading Time	Confidence Scores		
			Overall	\leq Age 4	$>$ Age 4
Dorsal spines	14.8	2.0 ± 0.3	2.8 ± 0.2	3.0 ± 0.2	2.6 ± 0.3
Scales	14.3	1.8 ± 0.2	3.0 ± 0.2	3.6 ± 0.2	1.9 ± 0.2
Whole otoliths	0.0	1.7 ± 0.2	3.0 ± 0.2	3.6 ± 0.2	2.2 ± 0.2
Sectioned otoliths	16.7	1.7 ± 0.2	4.1 ± 0.1	4.3 ± 0.1	3.7 ± 0.2

with age. Sectioned otoliths had by far the highest overall confidence scores, with an average of 4.1 (Table 6), significantly higher than all other structures ($P < 0.001$). Whole otoliths, scales and dorsal spines had lower overall confidence scores, ranging from 2.8 to 3.0, and they were not significantly different from one another ($P > 0.381$). Confidence scores were higher at younger ages (\leq age 4) than at older ages ($>$ age 4) for all structures, indicating a decrease in mark clarity at older ages (Table 6). Sectioned otoliths had by far the highest confidence 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 ($P \leq 0.001$). Scales had the lowest confidence scores at older ages (1.9), followed by whole otoliths (2.2) and dorsal spines (2.6). The low confidence scores for scales, whole otoliths, and dorsal spines indicate that marks on these structures were more difficult to interpret than those on sectioned otoliths. Indeed, the lowest average for sectioned otoliths (3.7 in fish $>$ age 4) was higher than the highest average (3.6) for all other structures at any age.

Agreement in age determinations for the same structure:

Within reader agreement (precision) in repeated age determinations varied between calcified structures. Complete within reader agreement was highest for sectioned otoliths, at 86% (Table 7). Complete agreement was somewhat lower in whole otoliths (74%) and was lowest for scales (67%) and dorsal spines (63%). Most within reader disagreements were within one year of each other, however, and within reader agreement increased to 100% for sectioned otoliths and to over 90% for whole otoliths, scales and dorsal spines when differences of one year were allowed.

Table 7. Average within and between reader percent agreement for presumed annual mark counts on bluefish calcified structures, expressed as complete agreement and agreement ± 1 year.

Structure	Within Reader Agreement		Between Reader Agreement	
	Complete	± 1 year	Complete	± 1 year
Dorsal Spines	63	92	38	77
Scales	67	97	62	97
Whole otoliths	74	91	62	85
Sectioned otoliths	86	100	83	97

Within reader precision in repeated age determinations also varied with age. For all structures, within reader complete agreement was highest by far at younger ages (Table 8, \leq age 4). It then decreased greatly after age 4. Sectioned otoliths showed the highest agreement at younger ages (95%), followed by whole otoliths (88%), scales (83%) and dorsal spines (71%). Sectioned otolith agreement remained the highest by far for fish over both age 4 (72%) and over age 7 (67%). Scales had the lowest agreement by far for fish over age 4 (33%), and agreement plunged to only 20% past age 7.

Between reader agreement varied among calcified structures and was generally lower than within reader agreement. Agreement between readers was highest by far for sectioned otoliths, at 83% (Table 7). Agreement was much lower for whole otoliths and scales (62%). It was lowest by far for dorsal spines (38%), reflecting the overall poor mark clarity of dorsal spines compared to other structures. When differences of one year were allowed, between reader percent agreement increased to 97% for sectioned otoliths and scales, but to only 85% for whole otoliths and 77% for dorsal spines.

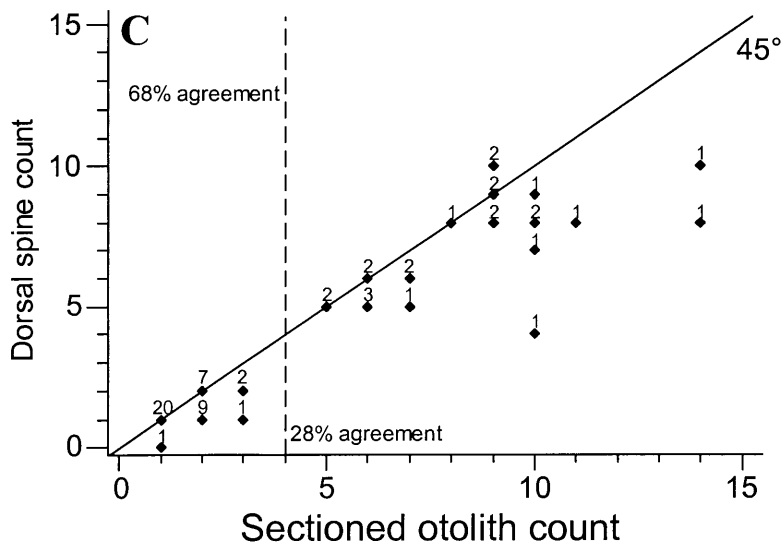
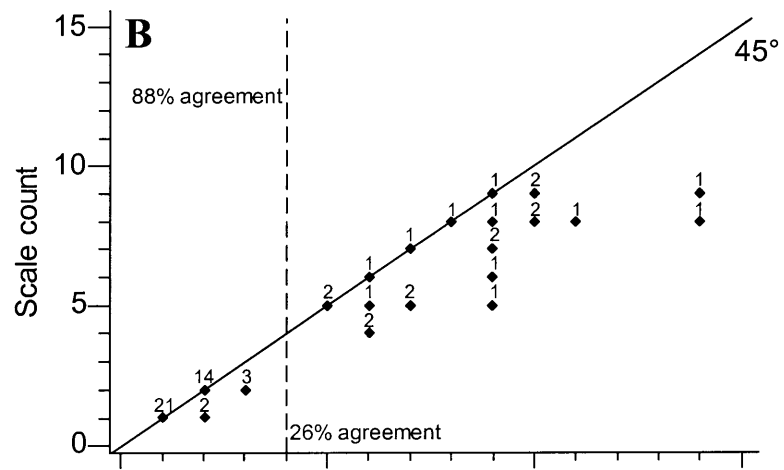
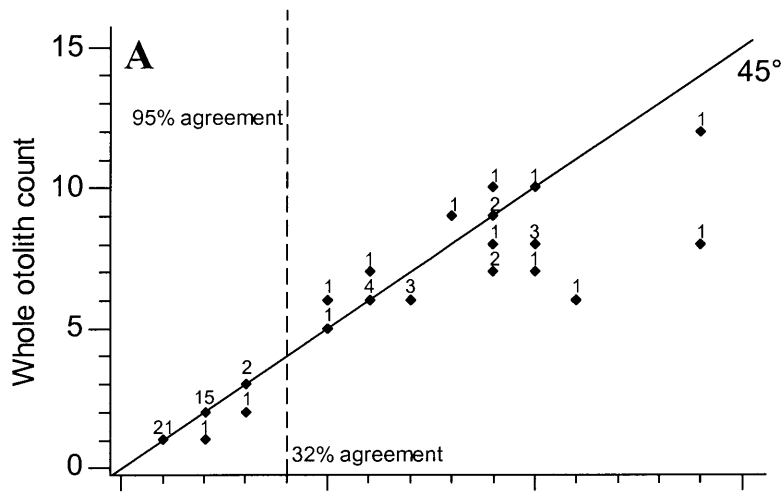
Comparison of different calcified structures from the same fish:

Different structures from the same bluefish often did not give the same estimate of presumed age. For all structure comparisons, the null hypothesis that the slope of the regression line equals one was rejected ($P < 0.001$), indicating that scales, whole otoliths and dorsal spines all gave different ages than sectioned otoliths (Figure 15), the structure with the highest confidence and within and between reader agreement. Between structure percent agreement varied with age, with all structures showing much higher agreement with sectioned otoliths at younger ages (68 to 95%) than at older ages (26 to 32%).

Table 8. Average within reader complete percent agreement by age for presumed annual mark counts on bluefish calcified structures. n = sample size.

Structure	\leq Age 4	n	$>$ Age 4	n	$>$ Age 7	n
Dorsal Spines	71	41	50	24	38	13
Scales	83	42	33	21	20	10
Whole otoliths	88	40	52	25	45	11
Sectioned otoliths	95	40	72	25	67	15

Figure 15. Comparisons of presumed annual mark counts on whole otoliths (A), scales (B), and dorsal spines (C) with mark counts on sectioned otoliths in bluefish. The 45° diagonal line represents 1:1 agreement. The number of fish is indicated at each symbol. Percent agreement between structures is indicated for fish \leq age 4 and for fish $>$ age 4.



Whole and sectioned otoliths showed the highest agreement in the number of presumed annual marks, at 71% complete agreement and 86% agreement within \pm one year. Complete agreement was very high for fish under age 4 (95%), but it decreased to only 32% in fish over age 4 (Fig. 15A). Whole otolith ages were generally lower than sectioned ages in older fish that disagreed, largely due to the opaque nature and faintness of edge marks that whole otoliths exhibited at older ages.

Scales and sectioned otoliths showed the second highest agreement in the number of presumed annual marks, at 65% complete agreement and 79% agreement within \pm one year. Complete agreement was high at younger ages (88%), but it decreased to only 26% past age 4 (Fig. 15B). Scale ages were lower than sectioned otolith ages in older fish that disagreed, largely due to mark crowding at the edge of scales and the thickening that was characteristic of scales from older fish.

Dorsal spines and sectioned otoliths showed the lowest agreement in the number of presumed annual marks, at only 52% complete agreement. Percent agreement within \pm one year was higher, at 86%, largely reflecting disagreements in young fish. Complete agreement was only 68% in fish under age 4 (Fig. 15C), but it increased to 98% within \pm one year. Sectioned otoliths aged higher than dorsal spines in all disagreements in younger fish, likely due to difficulties in identifying the first mark on dorsal spines. Complete agreement between dorsal spines and sectioned otoliths plunged to only 28% in fish over age 4 (Fig. 15C). Dorsal spines aged lower than sectioned otoliths in most of the disagreements for older fish, probably a result of erosion of early marks and crowding of later marks on dorsal spine sections of larger fish.

Some fish were difficult to age using all of the structures, suggesting that

something in a particular fish's life history might result in poor mark clarity on all of its structures. Likewise, all of the structures were clear and relatively easy to interpret in some fish. In 9 of the 65 fish examined, 3 of 4 structures showed within reader disagreement. All but one of those 9 fish was over age 7 (sectioned otolith consensus age). Similar comments were noted for all of the structures in these disagreements. For example, in one particularly difficult fish, it was noted that both whole and sectioned otoliths had light edge marks, scales had crowded edge marks, and dorsal spines had both light and crowded edge marks. In another difficult fish, it was noted that both sectioned and whole otoliths had "double marks" and that dorsal spines had "many fine striations" and "poor separation between marks." These difficult to age fish had much lower confidence scores (1.3 to 2 times lower, depending on the structure) and much higher reading times (1.7 to 3 times higher) than other more easily aged fish.

Structure growth with fish growth:

All calcified structures grew in size as bluefish body length grew, indicating that each structure might be useful for back-calculation studies. All regressions of structure size on fish FL were significant at $p < 0.001$, and all slopes were positive (Fig. 16). Regression of dorsal spine section core area on fish FL was also significant ($p < 0.001$), however, suggesting that growth of the vascular core might obscure or erode early marks. Comparison of core area with total spine area indicated that the largest core area in fish over 800 mm FL was as large as the total spine area in fish less than 500 mm FL (Fig. 17). This indicates that growth of the vascular core in dorsal spines has the potential to erode early marks on that structure in larger bluefish. Regressions of structure size on

Figure 16. Structure size in relation to fork length of bluefish for sectioned otoliths, whole otoliths, scales and dorsal spine sections. Sample sizes (n) differ because some structures were fractured and could not be measured.

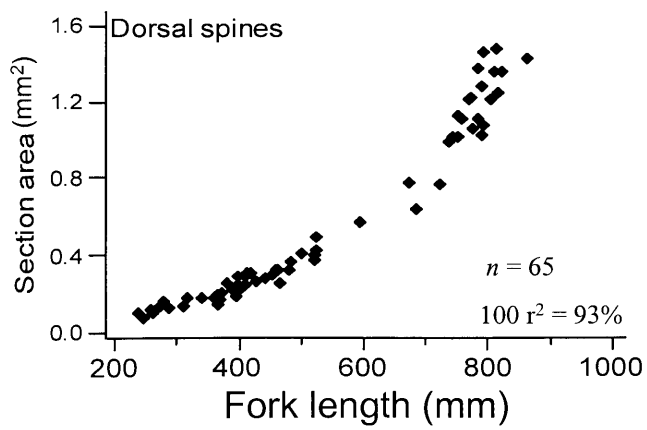
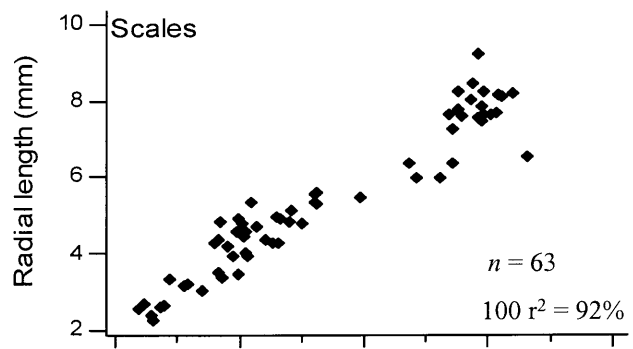
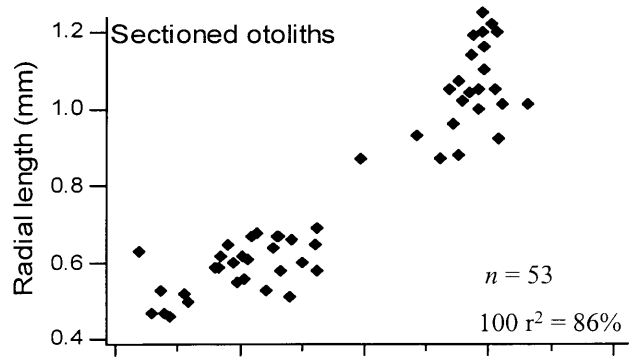
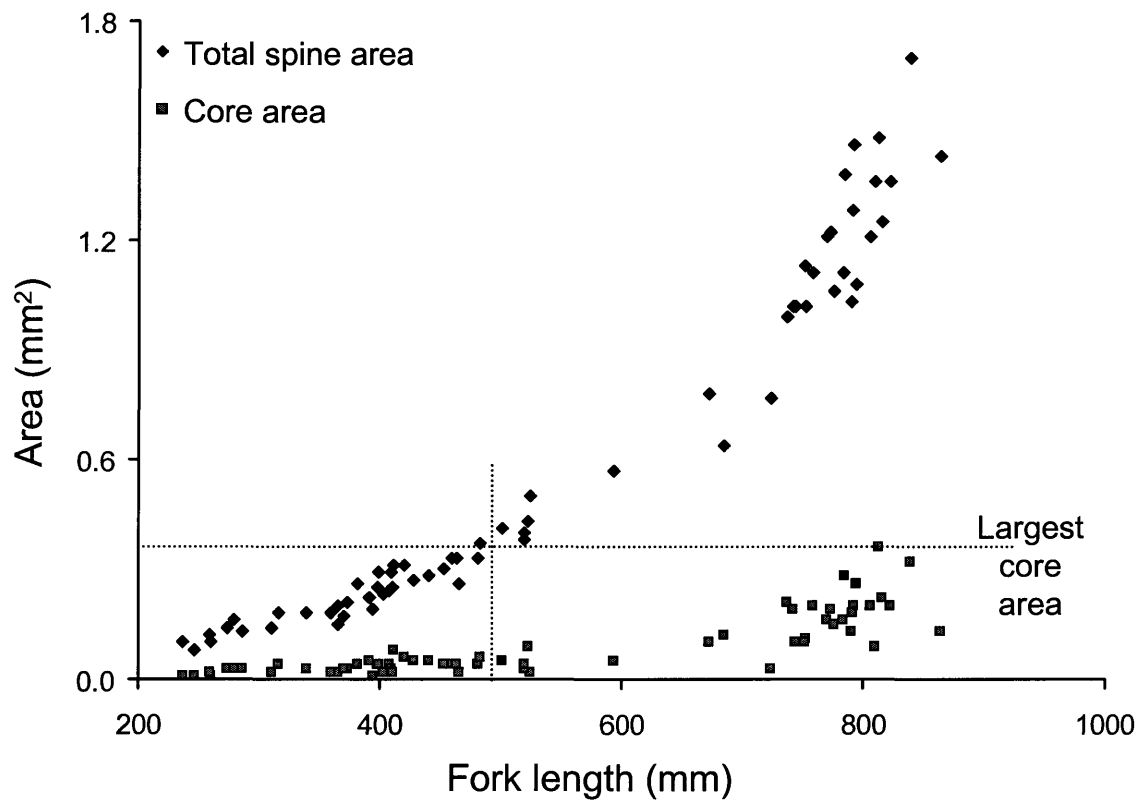


Figure 17. Dorsal spine section total area and vascular core area in relation to fork length in bluefish. The largest core area of dorsal spine sections from fish over 800 mm FL can exceed the total spine area of dorsal spine sections from fish less than 500 mm FL, indicating the potential for erosion of early marks by core growth in larger fish.



fish FL were generally strong and explained much of the variation in structure size, with coefficient of determination ($100r^2$) values of 86 to 93% (Fig. 16). Although some of the relationships between structure size and fish FL showed curvature, they were not tested for curvature because predictive ability was not important in these evaluations.

Increase in number of marks with structure size and fish size:

Mark counts on all calcified structures increased as structure size and fish size increased, indicating that each structure 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 (Fig. 18). Regressions were generally strong and explained much of the variation in mark counts, because $100r^2$ values were high, at 78 to 89%. Likewise, all regressions of mark counts on fish size were significant at $p < 0.001$, and all slopes were positive (Fig. 19). Although the relationships between mark count and fish FL showed curvature, as did some of the relationships between mark counts and structure size, they were not tested for curvature because predictive ability was not important in these evaluations. All regressions of mark counts on fish size were strong, with $100r^2$ values from 79 to 82%, values that would have increased if quadratic regressions were fitted.

Figure 18. Structure size in relation to mark counts for sectioned otoliths, whole otoliths, scales and dorsal spine sections of bluefish. Sample sizes (n) differ because some structures were fractured and could not be measured.

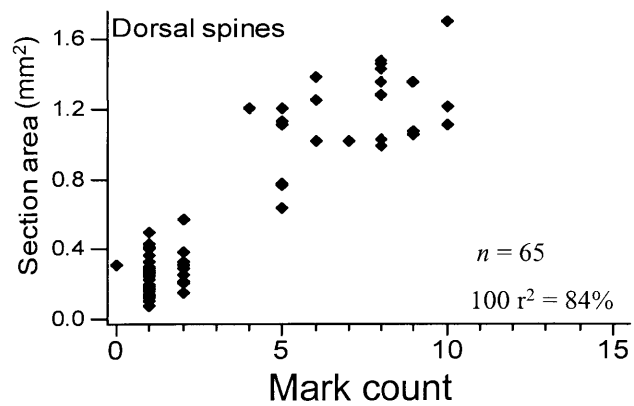
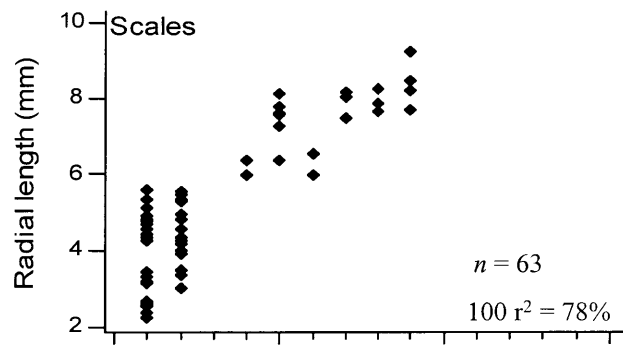
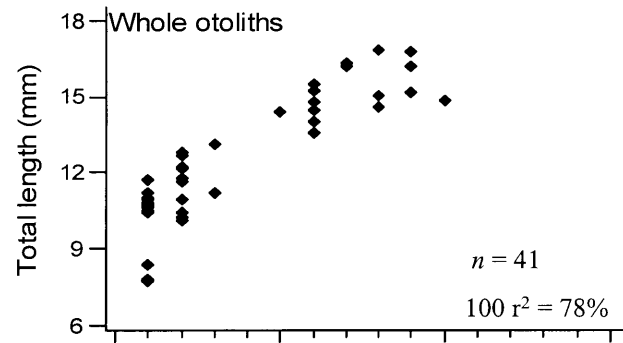
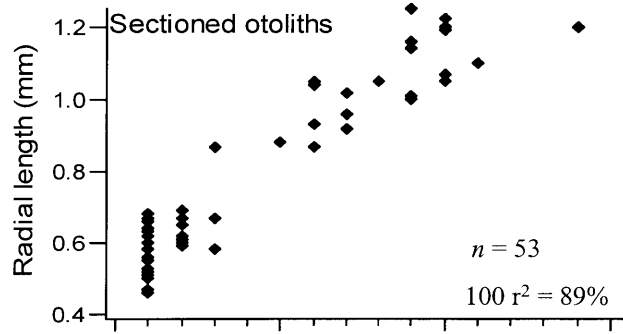


Figure 19. Fork length of bluefish in relation to mark counts of sectioned otoliths, whole otoliths, scales and dorsal spine sections. n = sample size.

Discussion

My initial evaluations of calcified structures indicated that neither opercular bones nor vertebrae sections were usable for aging bluefish, as neither structure showed any marks. No previous studies have examined opercular bones or vertebral sections for aging bluefish. Barger (1990) did examine vertebrae cut in half longitudinally, however he observed only 33% agreement between readers and, like me, chose not to use this structure for age determination.

My initial evaluations of dorsal spines, scales, and whole and sectioned otoliths indicated that each had potential for aging bluefish. All structures showed concentric marks that could be interpreted as annual and all grew in size as bluefish length grew. In addition, mark counts on each structure increased as structure size and fish size grew.

My comparisons indicated that sectioned otoliths are the best structure for aging bluefish over the age range 1 to 14 years. Sectioned otoliths were superior in all criteria used to evaluate bluefish calcified structures, despite some difficulties at older ages. Sectioned otoliths were consistently clearer and easier to interpret than whole otoliths, scales, and dorsal spines, and they had the highest within reader agreement for all ages, the highest between reader agreement, and the highest confidence scores both overall and at younger and older ages. These findings are largely new for bluefish because no previous studies have examined sectioned otoliths of bluefish in as great detail or over such a large age range. These findings agree, however, with many studies in other

species that have found sectioned otoliths to be the best aging structure (for examples, Chapter 2; Beamish, 1979; Chilton and Beamish, 1982; Beamish and McFarlane, 1983; Lowerre-Barbieri et al., 1994; Ihde, 2000).

My findings that sectioned otoliths are superior disagree with Barger (1990), who compared sectioned and whole otoliths in Gulf of Mexico bluefish. He chose to use whole otoliths based on the observation that otoliths fractured during sectioning and that mark spacing was too close on sectioned otoliths. In contrast, I did not find difficulty with close spacing of marks in sectioned otoliths of bluefish from the Chesapeake Bay region, and I found that problems with fracturing were alleviated with sectioning experience. Barger (1990) reported 70% agreement between sectioned and whole otoliths, similar to what I observed. However, he did not indicate the age range examined and did not report whether his disagreements were systematic or random, so it is unclear from his study whether whole or sectioned otoliths were better at older ages.

My comparisons indicated that whole otoliths are the second best structure for aging bluefish. Whole otoliths had no preparation time, the second highest within reader agreement, and the highest agreement with sectioned otoliths, especially at younger ages. Whole otoliths were generally easy to read in fish under age 4, and they are probably adequate at these younger ages. Marks on whole otoliths of larger fish were generally not as clear or as easy to read as those on sectioned otoliths, however, because whole otoliths became increasingly opaque at older ages. Indeed, whole otoliths often underaged compared to sectioned otoliths at older ages.

My findings on whole otolith 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 bluefish, and no studies have examined whole otoliths north of South Carolina (Barger, 1990). Barger (1990) found whole otoliths from Gulf of Mexico bluefish to have higher between reader agreement than scales and vertebrae. However, he did not examine within reader precision and did not report the age range of his fish, so it is unclear at what ages whole otoliths were acceptable for aging his fish. Barger (1990) also used whole otoliths to age bluefish captured from Florida to South Carolina, but he made no formal comparisons of whole otoliths to other structures in these fish.

Scales were inferior to, and much less desirable than, both sectioned and whole otoliths for aging bluefish. Marks on scales were often difficult to interpret using objective aging criteria, false marks and regenerated scales were common, and different scales from the same fish often gave different ages. As a result, both within and between reader percent agreement, agreement with sectioned otolith age, and confidence scores were undesirably low in scales, especially in fish over age 4. In addition, crowding of marks at the scale edge caused older fish to be underaged using scales. Therefore, I recommend that scales not be used for aging bluefish unless otoliths are not available.

The difficulties I found with bluefish scales generally agree with reports in the literature. Backus (1962), Lassiter (1962), and Richards (1976), for examples, reported similar problems interpreting scale marks in this species. Richards (1976) found that regenerated scales were common, that many scales had false annuli, and like Backus (1962), she found that ages sometimes varied between scales from the same fish. Lassiter (1962) 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.” His comment on opacity appears similar to my description of thickened and pitted scales at older ages. Barger (1990) and Chiarella and Conover (1990) are the only studies that report agreement in repeated scale age readings. Barger (1990) found only 67% between reader scale agreement, similar to the 62% I found. Chiarella and Conover (1990) reported higher agreement (89%) in repeated scale readings, but it is not clear what ages they used. I saw similarly high agreement in scales from younger fish. Chiarella and Conover (1990) also reported 93% agreement between scales and sectioned otoliths for fish up to age 6, similar to the 95% agreement I found for ages 0 to 4. However, they did not examine older fish. Their agreement may have decreased at older ages, since I found only 26% agreement between scales and sectioned otoliths past age 4.

Dorsal spine sections were inferior to all other structures examined for aging bluefish. They had the lowest within and between reader agreement, the lowest overall confidence scores, and the highest reading times. They often exhibited unclear transitions from translucent to opaque zones, and growth of the vascular core probably erodes early marks in older fish, leading to underestimation of age. Because of their many problems, I feel that dorsal spine sections should not be used for aging this species. My findings on dorsal spine section agreement, confidence scores, and reading times are new for bluefish, because no previous studies have used dorsal spine sections to age this species. Growth of the vascular core has been reported, however, for dorsal spine sections in other species (Hill et al., 1989; Gaichas, 1997).

Interpretation of marks on bluefish structures was often difficult, as indicated by the higher reading times, lower confidence scores, and lower structure agreement compared to that reported for other more easily aged species, such as summer flounder,

Paralichthys dentatus (Chapter 2), weakfish, *Cynoscion regalis* (Lowerre-Barbieri et al., 1994), and spotted seatrout, *Cynoscion nebulosus* (Ihde, 2000). For examples, bluefish sectioned otoliths took six times longer, whole otoliths took four times longer, and scales took 1.5 times longer to read than the same structures in summer flounder (Chapter 2). Similarly, I found only 86% within and 83% between reader agreement for sectioned otoliths in bluefish, whereas spotted seatrout (Ihde, 2000) and summer flounder (Chapter 2) had 100% and 97% within reader agreement, respectively, and 100% and 96% between reader agreement, respectively. The difficulties I encountered with bluefish structures closely resemble those of the more difficult to age Spanish mackerel, *Scomberomorus maculatus* (Gaichas, 1997), which also exhibited low confidence scores and problems with structure agreement. Sectioned otoliths, the structure that Gaichas (1997) found the best for aging Spanish mackerel, had only 79% within and 59% between reader agreement, the highest agreement of all the structures she examined.

Although I have found that sectioned otoliths are the best structure for determining bluefish age, my study has not proven their accuracy by validating the sectioned otolith method. However, I feel there is sufficient evidence to recommend that sectioned otoliths replace the current practice of using scales to age bluefish until validation studies are done.

Appendix

Maximum age and mortality estimates for bluefish, *Pomatomus saltatrix*:

Maximum age for bluefish in the Chesapeake Bay region was determined from sectioned otoliths of the 10 largest fish used in the comparison of calcified structures (Chapter 3). Total mortality rates (Z) were estimated from maximum age using Hoenig's (1983) equation for fish:

$$\ln(Z) = 1.46 - (1.01) * \ln t_{\max},$$

and Royce's (1972) equation:

$$Z = 4.6 / t_{\max},$$

where t_{\max} = maximum observed age. A maximum age of 14 years was observed in two of the largest fish in my sample, giving estimates of $Z = 0.30$ and 0.33 using Hoenig's (1983) and Royce's (1972) equation, respectively. The next highest age observed was 11 years in one fish, giving estimates of $Z = 0.38$ and 0.42 using Hoenig's (1983) and Royce's (1972) equation, respectively.

Literature Cited

- Able, K. W., R. E. Matheson, W. W. Morse, M. P. Fahay, and G. Shepherd. 1990. Patterns of summer flounder *Paralichthys dentatus* early life history in the Mid-Atlantic Bight and New Jersey estuaries. Fish. Bull., U.S. 88:1-12.
- Almeida, F. P., R. E. Castaneda, R. Jesien, R. E. Greenfield, and J. M. Burnett. 1992. Proceedings of the NEFC/ASMFC summer flounder, *Paralichthys dentatus*, aging workshop, 11-13 June 1990, Northeast Fisheries Center, Woods Hole, Massachusetts, NOAA Tech. Mem. NMFS-F/NEC-89, 7 p.
- Aschenbach, E. F., III. 1997. Growth and life history characteristics of the Pamunkey River, Virginia population of American shad, *Alosa sapidissima*. MS Thesis, Virginia Commonwealth University, Richmond, Virginia, 47 p.
- Babaluk, J. A. and J. S. Campbell. 1987. Preliminary results of tetracycline labeling for validating annual growth increments on the opercula of walleyes. N. Amer. J. Fish. Mgmt. 7:138-141.
- 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. In Methods for assessment of fish production in fresh waters. 3rd edition. T. B. Bagenal, editor. Blackwell Scientific Publications, Oxford, 101-136.

- Barbieri, L. R., M. E. Chittenden, Jr., and C. M. Jones. 1994a. Age, growth, and mortality of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay region, with a discussion of apparent geographic changes in population dynamics. Fish. Bull., U.S. 92:1-12.
- Barbieri, L. R., M. E. Chittenden, Jr., and S. K. Lowerre-Barbieri. 1994b. Maturity, spawning, and ovarian cycle of Atlantic croaker, *Micropogonias undulatus*, in the Chesapeake Bay and adjacent coastal waters. Fish. Bull., U.S. 92:671-685.
- Bardach, J. E. 1955. The opercular bone of the yellow perch, *Perca flavescens*, as a tool for age and growth studies. Copeia 2:107-109.
- Barger, L. E. 1990. Age and growth of bluefish *Pomatomus saltatrix* from the Northern Gulf of Mexico and U.S. South Atlantic coast. Fish. Bull., U.S. 88:805-809.
- Beamish, R. J. 1979. Differences in the age of Pacific hake (*Merluccius productus*) using whole otoliths and sections of otoliths. J. Fish. Res. Bd. Canada 36:141-151.
- Beamish, R. J. and D. E. Chilton. 1982. Preliminary evaluation of a method to determine the age of sablefish (*Anoplopoma fimbria*). Can. J. Fish. Aquat. Sci. 39:277-287.
- Beamish, R. J. and G. A. McFarlane. 1983. The forgotten requirement for age validation in fisheries biology. Trans. Amer. Fish. Soc. 112:735-743.
- Bigelow, H. B. and W. C. Schroeder. 1953. Fishes of the Gulf of Maine. U.S. Fish Wildl. Serv., Fish. Bull. 53:1-577.

- Bolz, G. R., J. P. Monaghan, Jr., K. L. Lang, R. W. Gregory, and J. M. Burnett. 2000. Proceedings of the summer flounder aging workshop, 1-2 February 1999, Woods Hole, Massachusetts. NOAA Tech. Mem. NMFS-NE-156, 15 p.
- Briggs, J. C. 1960. Fishes of worldwide (circumtropical) distribution. *Copeia* 1960:171-180.
- Cailliet, G. M., L. K. Martin, D. Kusher, P. Wolf, and B. A. Welden. 1983. Techniques for enhancing vertebral bands in age estimation of California elasmobranchs. NOAA Tech. Rep. NMFS 8:157-165.
- Casselman, J. M. 1983. Age and growth assessment of fish from their calcified structures - techniques and tools. NOAA Tech. Rep. NMFS 8:1-17.
- Chiarella, L. A. and D. O. Conover. 1990. Spawning season and first-year growth of adult bluefish from the New York Bight. *Trans. Amer. Fish. Soc.* 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. *Can. Spec. Publ. Fish. Aquat. Sci.* 60:1-102.
- Cooper, R. A. 1967. Age and growth of the tautog, *Tautoga onitis* (Linnaeus), from Rhode Island. *Trans. Amer. Fish. Soc.* 96:134-142.
- Dery, L. M. 1988. Summer flounder, *Paralichthys dentatus*. In Age determination methods for Northwest Atlantic species. J. Penttila and L. M. Dery, editors. NOAA Tech. Rep. NMFS 72, 135 p.

- Desfosse, J. C. 1995. Movements and ecology of summer flounder, *Paralichthys dentatus*, tagged in the southern Mid-Atlantic Bight. PhD Diss., College of William and Mary, Williamsburg, Virginia, 187 p.
- Devries, D. R. and R. V. Frie. 1996. Determination of age and growth. *In* Fisheries techniques. B. R. Murphy and D. W. Willis, editors. American Fisheries Society, Bethesda, Maryland, 483 – 512.
- Diagnostic Instruments, Inc. 1999. User Guide to the SPOT RT software, 232 p.
- Donald, D. B., J. A. Babaluk, J. F. Craig, and W. A. Musker. 1992. Evaluation of scales and operculum methods to determine age of adult goldeyes with special reference to a dominant year-class. *Trans. Amer. Fish. Soc.* 121:792-796.
- Eldridge, P. J. 1962. Observations on the winter trawl fishery for summer flounder, *Paralichthys dentatus*. MS Thesis, College of William and Mary, Williamsburg, Virginia, 58 p.
- Frost, W. E. and C. Kipling. 1957. The determination of the age and growth of pike (*Esox lucius* L.) from scales and opercular bones. *J. Cons.* 24:314-341.
- Gaichas, S. K. 1997. Age and growth of Spanish mackerel, *Scomberomorus maculatus*, in the Chesapeake Bay region. MS Thesis, College of William and Mary, Williamsburg, Virginia, 111 p.
- Ginsburg, I. 1952. Flounders of the genus *Paralichthys* and related genera in American waters. U.S. Fish Wildl. Serv., Fish. Bull. 52:267-351.

- Gutherz, E. J. 1967. Field guide to the flatfishes of the Family Bothidae in the western North Atlantic. U.S. Fish Wildl. Serv. Circ. 263, 47 p.
- Hamer, P. E. 1959. Age and growth studies of the bluefish (*Pomatomus saltatrix* Linnaeus) of the New York Bight. MS Thesis, Rutgers University, New Brunswick, New Jersey, 27 p.
- Haven, D. S. 1957. Distribution, growth, and availability of juvenile croaker, *Micropogon undulatus*, in Virginia. Ecology 38:88-97.
- Hildebrand, S. F. and W. C. Schroeder. 1928. Fishes of Chesapeake Bay. Bull. U.S. Bur. Fish 43:1-366.
- 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*. Fish. Bull., U.S. 87:829-843.
- Hoenig, J. M. 1983. Empirical use of longevity data to estimate mortality rates. Fish. Bull., U.S. 82:898-902.
- Hostetter, E. B. and T. A. Munroe. 1993. Age, growth, and reproduction of tautog *Tautoga onitis* (Labridae:Perciformes) from coastal waters of Virginia. Fish. Bull., U.S. 91:45-64.
- Ihde, T. F. 2000. Biology of the spotted seatrout, *Cynoscion nebulosus*, in the Chesapeake Bay region. MS Thesis, College of William and Mary, Williamsburg, Virginia, 121 p.

- Johnson, A. G. 1979. A simple method for staining the centra of teleost vertebrae. *Northeast Gulf Sci.* 3:113-115.
- Lai, H. L., D. R. Gunderson, and L. L. Low. 1987. Age determination of Pacific cod, *Gadus macrocephalus*, using five ageing methods. *Fish. Bull., U.S.* 85:713-722.
- Lassiter, R. R. 1962. Life history aspects of the bluefish, *Pomatomus saltatrix* (Linnaeus) from the coast of North Carolina. MS Thesis, North Carolina State College, Raleigh, North Carolina, 102 p.
- LeCren, E. D. 1947. The determination of the age and growth of the perch (*Perca fluviatilis*) from the opercular bone. *J. Anim. Ecol.* 16:188-204.
- Leim, A. H. and W. B. Scott. 1966. Fishes of the Atlantic Coast of Canada. *Fish. Res. Bd. Canada, Bull.* 155:1-485.
- 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. *Fish. Bull., U.S.* 92:555-568.
- MAFMC (Mid-Atlantic Fishery Management Council). 1987. Fishery management plan for the summer flounder fishery, 159 p.
- MAFMC (Mid-Atlantic Fishery Management Council). 1990. Fishery management plan for the bluefish fishery, 81 p.
- McConnell, W. J. 1952. The opercular bone as an indicator of age and growth of the carp, *Cyprinus carpio* Linnaeus. *Trans. Amer. Fish. Soc.* 81:138-149.

- Merriman, D. 1941. Studies on the striped bass (*Roccus saxatilis*) of the Atlantic coast. Fish. Bull., U.S. 50:1-77.
- Morse, W. W. 1981. Reproduction of the summer flounder, *Paralichthys dentatus* (L.). J. Fish. Biol. 19:189-203.
- Poole, J. C. 1961. Age and growth of the fluke in Great South Bay and their significance to the sport fishery. N.Y. Fish Game J. 8:1-18.
- Poole, J. C. 1962. The fluke population of Great South Bay in relation to the sport fishery. N.Y. Fish Game J. 9:93-117.
- Powell, A. B. 1982. Annulus formation on otoliths and growth of young summer flounder from Pamlico Sound, North Carolina. Trans. Amer. Fish. Soc. 111:688-693.
- Richards, S. W. 1976. Age, growth, and food of bluefish (*Pomatomus saltatrix*) from East-Central Long Island Sound from July through November 1975. Trans. Amer. Fish. Soc. 105:523-525.
- Royce, W. F. 1972. Introduction to the fishery sciences. Academic Press, New York, 351 p.
- Scofield, E. C. 1931. The striped bass of California (*Roccus lineatus*). Calif. Fish Game Bull. 29:1-84.
- Secor, D. H., T. M. Trice, and H. T. Hornick. 1995. Validation of otolith-based ageing and a comparison of otolith and scale-based ageing in mark-recaptured

- Chesapeake Bay striped bass, *Morone saxatilis*. Fish. Bull., U.S. 93:186-190.
- Sharp, D. and D. R. Bernard. 1988. Precision of estimated ages of lake trout from five calcified structures. N. Amer. J. Fish. Mgmt. 8:367-372.
- Shepherd, G. 1980. A comparative study of ageing methods for summer flounder (*Paralichthys dentatus*). NEFC, NMFS Woods Hole Lab. Ref. Doc. No. 80-13, 26 p.
- Smith, R. W. and F. C. Daiber. 1977. Biology of the summer flounder, *Paralichthys dentatus*, in Delaware Bay. Fish. Bull., U.S. 75:823-830.
- Smith, R. W., L. M. Dery, P. G. Scarlett, and A. Jearld, Jr. 1981. Proceedings of the summer flounder (*Paralichthys dentatus*) age and growth workshop, 20-21 May 1980, NEFC, Woods Hole, Massachusetts. NOAA Tech. Mem. NMFS-F/NEC-11, 30 p.
- Smith, W. G. 1973. The distribution of summer flounder, *Paralichthys dentatus*, eggs and larvae on the continental shelf between Cape Cod and Cape Lookout. Fish. Bull., U.S. 71:527-535.
- Soupir, C. A., B. B. Blackwell, and M. L. Brown. 1997. Relative precision among calcified structures for white bass age and growth assessment. J. Fresh. Ecol. 12:531-538.
- Szedlmayer, S. T., K. W. Able, and R. A. Rountree. 1992. Growth and temperature induced mortality of young-of-the-year summer flounder (*Paralichthys dentatus*)

- in southern New Jersey. *Copeia* 1992:120-128.
- 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*). *Fish. Bull., U.S.* 91:534-549.
- Vilizzi, L. and K. F. Walker. 1999. Age and growth of the common carp, *Cyprinus carpio*, in the River Murray, Australia: validation, consistency of age interpretation, and growth models. *Env. Biol. Fish.* 54:77-106.
- Wallace, D. H. 1940. Sexual development of the croaker, *Micropogon undulatus*, and distribution of the early stages in Chesapeake Bay. *Trans. Amer. Fish. Soc.* 70:475-482.
- Wenner, C. A., W. A. Roumillat, J. E. Moran, Jr., M. B. Maddox, L. B. Daniel, and J. W. Smith. 1990. Investigations of the life history and population dynamics of marine recreational fishes in South Carolina: part 1. South Carolina Wildl. Mar. Resour. Dep., Mar. Resour. Res. Instit., 26 p.
- Wilk, S. J. 1977. Biological and fisheries data on bluefish, *Pomatomus saltatrix* (Linnaeus). NMFS, NEFC, Sandy Hook Lab. Tech. Ser. Rep. 11, Highlands, New Jersey, 56 p.
- Wright, R. M. and N. Giles. 1991. The population biology of tench, *Tinca tinca* (L.), in two gravel pit lakes. *J. Fish. Biol.* 38:17-28.
- Zar, J. H. 1996. Biostatistical analysis. third edition. Prentice Hall, New Jersey, 662 p.

VITA

Ann Marie Sipe

Born in Hanover, Pennsylvania on May 14, 1973. Graduated from York Catholic High School in York, PA in 1991. Entered Drexel University in Philadelphia, PA in September 1991. Worked as a microbiologist/analytical chemist at Environmental Research Laboratories of SmithKline Beecham Pharmaceuticals in Conshohocken, PA, 1993 to 1995, and as a biochemist in the Drug Disposition and Metabolism department at Zeneca Pharmaceuticals in Wilmington, DE, 1994 to 1997. Participated in 3 month SEA Semester program out of Woods Hole, MA in fall 1995. Graduated magna cum laude from Drexel University in June, 1996 with a B.S. in Environmental Science. Entered Masters program in Marine Science at the College of William and Mary, School of Marine Science, Virginia Institute of Marine Science in August 1997. Defended Masters thesis on April 25, 2001.