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A Comparison of a Validated Otolith Method to Age Weakfish, Cynoscion regalis, With the Traditional Scale Method

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Abstract.—Otoliths, scales, dorsal spines, and pectoral-fin rays were compared to ascertain the best hardpart for determining the age of weakfish, *Cynoscion regalis*. Each showed concentric marks, which could be interpreted as annuli. Sectioned otoliths, however, consistently showed the clearest marks, had 100% agreement between and within readers, and were validated by the marginal increment method for ages 1–5. This validated method of ageing weakfish was then compared with the traditionally used scale method. The scale method was less precise, as demonstrated by lower percent agreement, and generally assigned younger ages for fish older than age 6 (as determined by otoliths). Consequently, mean sizes at age based on scales showed no clear signs of an asymptote, whereas those based on otoliths did. Otolith annuli formed in April and May, whereas scale annulus formation was more variable, ranging from April to August. This extended time of annulus formation made scales poorly suited for back calculation.

A comparison of a validated otolith method to age weakfish, *Cynoscion regalis*, with the traditional scale method

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The weakfish, *Cynoscion regalis*, is a recreationally and commercially important sciaenid found from eastern Florida to Massachusetts, and is most abundant from North Carolina to New York (Mercer, 1985). Believed to be resident year-round in the Carolinas, they are found farther north only seasonally (Bigelow and Schroeder, 1953). In the spring, weakfish migrate northward and inshore to estuarine feeding and spawning grounds; this pattern is reversed in the fall (Wilk, 1979). Most fish are believed to overwinter off North Carolina (Pearson, 1932). Weakfish are found in Chesapeake Bay, roughly from April through November (Pearson, 1941; Massmann et al., 1958), where they support one of the region's most important fisheries (Rothschild et al., 1981).

Weakfish age and growth studies have been based almost exclusively on scales (Taylor, 1916; Nesbit, 1954; Perlmutter et al., 1956; Massmann, 1963a; Merriner, 1973; Shepherd and Grimes, 1983). However, problems with this method have been reported: 1) small fish may not lay down a first annulus on scales (Welsh and Breder, 1923), 2) older fish have closely spaced annuli that are difficult to interpret (Taylor, 1916; Shepherd, 1988), 3) annuli form over a long time period, April–August, and scales are difficult to interpret during annulus formation (Nesbit, 1954; Massmann, 1963b), 4) the time annuli form varies annually and regionally (Perlmutter et al., 1956), and 5) checks (false annuli) and regenerated scales are common (Merriner, 1973). The scale method of ageing weakfish also has not been conclusively validated by current standards (Beamish and McFarlane, 1983; Brothers, 1983). Perlmutter et al. (1956) and Shepherd and Grimes (1983) both tried to validate annuli on scales by the marginal increment method, however they used pooled age data and did not report the age range.

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Although recent studies have shown that for many species the scale method underages older fish at the point where fish growth becomes asymptotic (Beamish and Chilton, 1981; Beamish and McFarlane, 1983; Barnes and Power, 1984), there has been little evaluation of other weakfish hardparts. Merriner (1973) compared weakfish scales to whole vertebrae and otoliths, and Villoso (1989) compared scales to whole otoliths. Both concluded that scales were best. However, Merriner's study was conducted before thin-sectioning of otoliths (Williams and Bedford, 1974; Beamish, 1979; Beamish and Chilton, 1981) and other hardparts became common and Villoso (1989) did not consider thin-sectioning.

A decline in weakfish landings since 1980, coupled with greater competition between fisheries, caused the Atlantic States Marine Fisheries Commission (ASMFC) to develop a weakfish management plan in 1985 (Mercer, 1985). Since then the ASMFC has issued an updated stock assessment[1] and suggested a 25% reduction in coast-wide exploitation rates (Amendment No. 1 of the Weakfish Fishery Management Plan of the ASMFC). However, it is essential to proper weakfish management that a validated ageing technique be developed and used, as improper ageing can lead to faulty estimates of model parameters such as age at maturity, growth, longevity and mortality (Beamish and McFarlane, 1983).

The objectives of this study were 1) to compare otolith, dorsal-fin spine, and pectoral-fin ray sections with scales in terms of legibility and interpretation of potential annual marks, ease of collection and processing, and precision, 2) to validate the hardpart demonstrating the greatest clarity by marginal increment analysis for each age group found in the Chesapeake Bay area, and 3) to conduct a more in-depth comparison of the validated hardpart with scales in terms of precision and accuracy, time of annulus formation, growth estimates, and use in back calculation of body length.

Methods

Preliminary comparison of hardparts

Four hundred weakfish were collected every other week during April–October in 1989 from three Chesapeake Bay commercial pound nets. On each collection day, one 22.7 kg (50 lb) box of each available grade of weakfish—small, medium, or large—was bought and all fish within it processed. Fish were measured for total length (TL ±1.0 mm), sexed, and both sagittal otoliths were removed and stored dry. Scales were removed from an area just posterior to the tip of the left pectoral fin, below the lateral line. The left pectoral fin and the entire dorsal fin were removed by cutting below the base of the rays. Scales and fins were stored in paper envelopes and kept frozen until preparation for ageing.

A total of 45 fish, 15 from each grade, were randomly selected from the fish collected in 1989 for a preliminary comparison of hardparts. These fish ranged from 244 to 615 mm TL and each of their four hardparts was prepared for reading as described below.

The right otolith from each fish was transversely sectioned through the nucleus with a Buehler low-speed Isomet saw. Sections 350–500 µm thick were mounted on glass slides with Flo-Texx clear mounting medium and viewed under a dissecting microscope at 24× magnification by using transmitted light and bright field, with the exception of samples from the period April–May, when sections were also read with reflected light and dark field to help identify the last annulus. Thin opaque bands, presumed to represent annual marks, were counted along the otolith sulcal groove (Fig. 1). Because opaque bands inhibit light passage, they appeared dark in transmitted light (Fig. 2A) and light in reflected light.

Scales from each fish were soaked in water until soft, after which they were washed gently with a soft-bristled tooth brush. Three or four clean, unregenerated scales

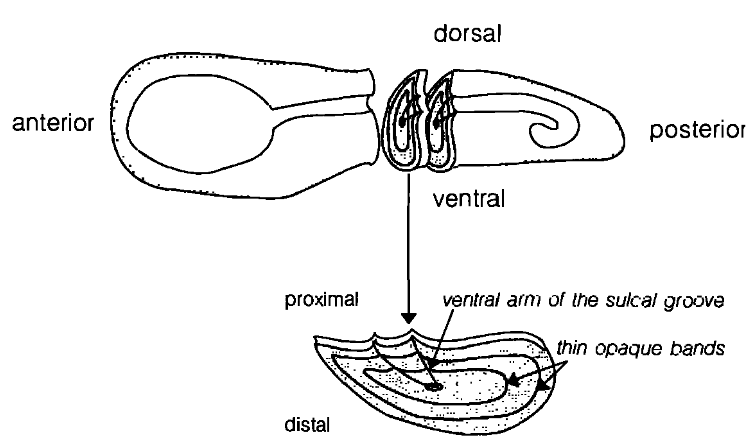

Figure 1

Schematic representation of a transverse section taken through the right sagittal otolith. The ventral arm of the sulcal groove, along which otoliths were measured, is indicated. The whole otolith is positioned as it would be in a weakfish, *Cynoscion regalis*.

[1] Vaughan, D. S., R. J. Seagraves, and K. West. 1991. An assessment of the Atlantic weakfish stock, 1982–1988. Atl. States Mar. Fish. Comm. Spec. Rep. 21, Wash. DC, 29 p.

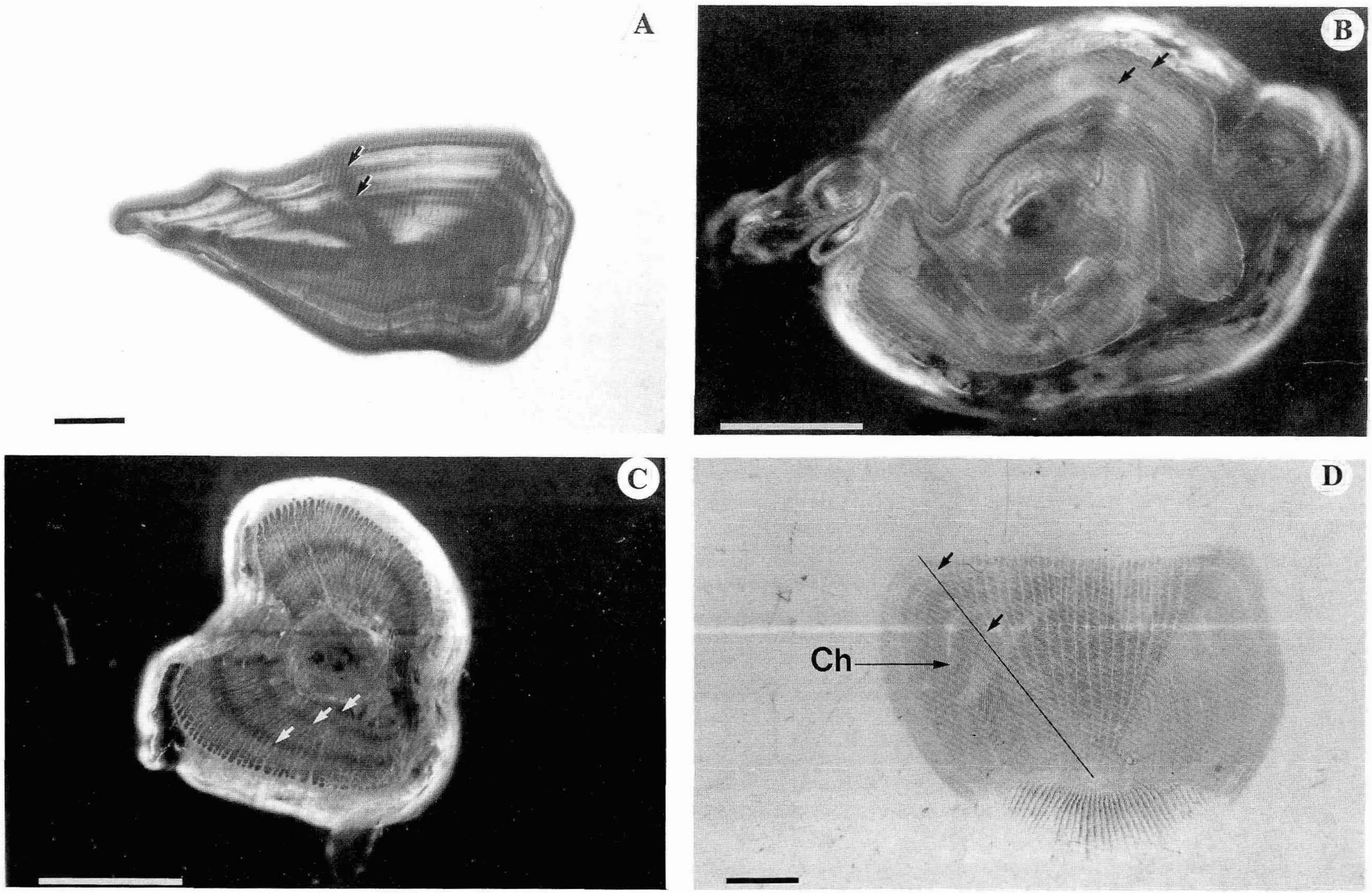

Figure 2

Marks on hardparts taken from a two-year-old (as aged by otoliths) female weakfish, *Cynoscion regalis*, TL=392 mm, collected in mid-September. (**A**) otolith section, as seen in transmitted light, bar=1 mm; (**B**) pectoral ray section, as seen in transmitted light and dark field, bar=0.5 mm; (**C**) dorsal spine section, as seen in transmitted light and dark field, bar=0.5 mm; and (**D**) scale impression, as seen in transmitted light, bar=1 mm. The left radius, which was the scale measuring axis, is marked. Ch=check. Arrows indicate individual marks counted.

were then dried, taped to an acetate sheet, inserted between two other blank sheets, and pressed with a Carver laboratory scale press for two minutes at 2,721 kg of pressure at 71°F. Because of the large size of weakfish scales, scale impressions were read with a standard microfiche reader at 20×. Those scales with potential annuli crowded along the scale periphery were also viewed at 48× under a dissecting microscope. Presumed annual marks were identified by standard criteria (Bagenal and Tesch, 1978; Shepherd, 1988).

One spiny ray from the dorsal fin and one soft ray of the left pectoral fin were prepared from each fish. Rays were serially sectioned by starting at their base and cutting through most of their length at a thickness of 400 μm with a Buehler low-speed Isomet saw. Sections were then mounted on microscope slides with Flo-Texx and read under a dissecting microscope with transmitted light and dark field at 64×. Presumed annual marks were counted when they could be identified as individual, opaque bands.

Each hardpart was read twice by two separate readers. Readings were done in a randomly selected order, with no knowledge of collection date or fish size. Hardparts were evaluated in terms of clarity of presumed annual marks, ease of collection and processing, and precision. Precision was measured by average percent agreement within and between readers, i.e. percent agreement within readers was calculated for each reader separately and then averaged for the two readers and percent agreement between readers was calculated separately for each reading and then averaged for the two readings.

Validation of the otolith method

Because otoliths were found best for ageing, additional samples were collected for validation. During 1989–92, 1,928 weakfish were collected from commercial pound-net, haul-seine, and gill-net fisheries in Chesapeake Bay. During March–November when weakfish are not present in the Chesapeake Bay, fish were collected (n=289) from the trawl fishery operating in North Carolina shelf waters north of Cape Hatteras.

The marginal increment method was used to validate otolith annuli (Brothers, 1983; Casselman, 1987; Hyndes et al., 1992). The translucent margin outside the proximal end of the last annulus was measured along the ventral side of the otolith sulcal groove (Fig. 1). Measurements were taken with an ocular micrometer to the nearest 0.038 mm (one micrometer unit at a total magnification of 24×).

Comparison of scales and otoliths

To compare the otolith and scale methods in more detail, 155 fish ranging from 140 to 845 mm TL were selected by stratified, random subsampling—strata being otolith-determined ages—from a total of 300 fish collected in 1989 and 1992. Thirty fish were selected from each of the age-strata, 1–4. Because older fish were scarce, only 14 age-5, 16 age-6, two age-7, two age-8, and one age-10 fish were included. Although most fish came from Chesapeake Bay commercial fisheries, in order to increase the number of older fish, 27 fish were collected in May 1992 at the Delaware Bay Weakfish Sport Fishing Tournament. We collected an additional 20 fish in August 1992 to include fish from each of the summer months for marginal increment and back-calculation analyses.

Hardparts were prepared as described for the preliminary comparison and read twice by each of two readers. An effort was made to determine annuli on scales based only on physical criteria and not to assign annuli based on any preconceived ideas of growth (Casselman, 1983). Reading order was randomized and collection date and fish size were unknown. Each reader recorded the number of presumed annuli and a "+" if there was growth beyond the last annulus or a "*" if the last presumed annulus was forming or had just formed (Casselman, 1987). After all hardparts had been read, we assigned ages using a January 1 birthdate, knowledge of the time of annulus formation, the relative growth of the hardpart margin, and date of capture.

Variability within reader, between readers, and between hardparts was analyzed by percent agreement. When an individual reader's counts of presumed annuli disagreed, a third reading was made. When readers' ages disagreed, a third reading with both readers present was made to resolve the disagreement.

To compare time of annulus formation and its variability in scales and otoliths, mean monthly relative marginal increments and their ranges were calculated and plotted (April–October). Relative marginal increments were calculated by dividing the marginal increment by the hardpart radius. All ages were pooled. Additionally, those hardparts which had been designated as having an annulus on the margin ("*") were reviewed and their time of collection recorded.

To determine marginal increments and to conduct back-calculation analyses, hardparts were measured by using a Via 100 camera/monitor system with a dissecting microscope at 24×. Otolith radius (OR) and otolith annular radius (OAR)—the distance from the nucleus to the proximal edge of each annulus—were measured along the ventral arm of the sulcal groove. Scale radius (SR) and scale annular radius (SAR) were measured along the left radius (Ricker, 1992). Marginal growth was measured from outside the last annulus to the hardpart edge.

To evaluate the applicability of scales and otoliths for back-calculation, it was necessary to first analyze separately their total length to hardpart relationships. Seasonal effects were assessed by comparing hardpart size of one age class taken from different seasons to that predicted by the linear regression of total length on hardpart size for all fish. Only one age class (age 3) was used to remove any confounding effects of age. This age class was chosen because it was well-represented throughout the seasons.

Back-calculation relationships for both scales and otoliths were based on the "body proportional" hypothesis (Francis, 1990) proposed by Whitney and Carlander (1956):

$$L_i = [g\,(S_i) \,/\, g\,(S_c)]\, L_c,$$

where g is the total length on hardpart radius function, L_i is back-calculated TL at age i, S_i is the measured hardpart size at annulus i, and S_c and L_c are the respective hardpart size and total length at capture. Only fish collected in April and May—the beginning of the somatic growth season—were used, to remove seasonal effects from the back-calculation equations (Ricker, 1992). Because body-proportional back-calculation is based not just on the relationship of hardpart size to total length but also on the relationship of hardpart size to consecutive annuli, mean annual growth increments were also calculated and compared between scales and otoliths.

The tendency for older fish to produce smaller back-calculated lengths at younger ages than observed, known as Lee's phenomenon (Smith, 1983), was evaluated by calculating mean SAR and mean OAR for each age at capture. In this way it was possible to determine if older fish demonstrated slower hardpart growth at younger ages, i.e. true Lee's phenomenon (Smale and Taylor, 1987).

Data were analyzed by using χ^2 tests and regression methods available through the Statistical Analysis System (SAS 1988). Rejection of the null hypothesis in statistical tests was based on α=0.05. Assumptions of linear models were checked by residual plots as described in Draper and Smith (1981).

Results

Preliminary comparison of hardparts

All four hardparts showed concentric marks that were interpreted as annuli (Fig. 2). However, marks on the dorsal spines and pectoral rays were inconsistent, often blurred or impossible to follow around most of the section and therefore difficult to interpret. Presumed annuli on scales were distinctly clearer and more regular than those on dorsal spines and pectoral rays, but they still required some subjective interpretation. Presumed annuli on otoliths were exceptionally clear, consistent, and easy to interpret.

Typical otolith sections showed an opaque nucleus surrounded by a translucent zone followed by a pattern of thin, opaque zones alternating with wide, translucent zones along the sulcal groove (Fig. 2A). In some sections the translucent zone between the nucleus and the first opaque zone was relatively small and made more opaque by a number of fine, circular, opaque bands. However, in all sections the first opaque zone beyond the nucleus was easily identified and considered to be the first annulus.

Presumed annuli on scales were harder to identify than those on otoliths but were usually identifiable as a clear zone in the anterior field, where circuli are either absent or more widely spaced, and by cutting over in the lateral fields (Fig. 2D). Checks were most apparent in the anterior field. A clear zone in the anterior field was considered a check if it was not accompanied by distinct cutting over in the lateral fields. The first annulus was the hardest to identify. It rarely showed a clear band in the radii zone, although cutting over was sometimes apparent. Its position was based predominantly on the first point at which a large number of secondary radii originated.

Presumed annual marks on dorsal spines were fairly clear in some sections but incomplete or blurred in others (Fig. 2C), whereas pectoral-fin ray sections were consistently hard to interpret (Fig. 2B). Presumed annual marks on both these hardparts appeared as wide, opaque, semicircular bands alternating with narrow translucent zones.

Otoliths showed the greatest precision, with 100% average agreement within and between readers. Scales also had high average agreement: 89% within readers and 80% between readers. Dorsal and pectoral fin sections showed the lowest agreement (Table 1) and little confidence was attached to their age assignments.

Table 1

Average percent agreement in the preliminary comparison of weakfish, *Cynscion regalis*, hardpart mark counts within readers, between readers, and with otoliths.

Hardpart	Within readers	Between readers	With otoliths
Scales	89	80	27
Pectoral rays	59	64	49
Dorsal spines	66	76	46
Otoliths	100	100	

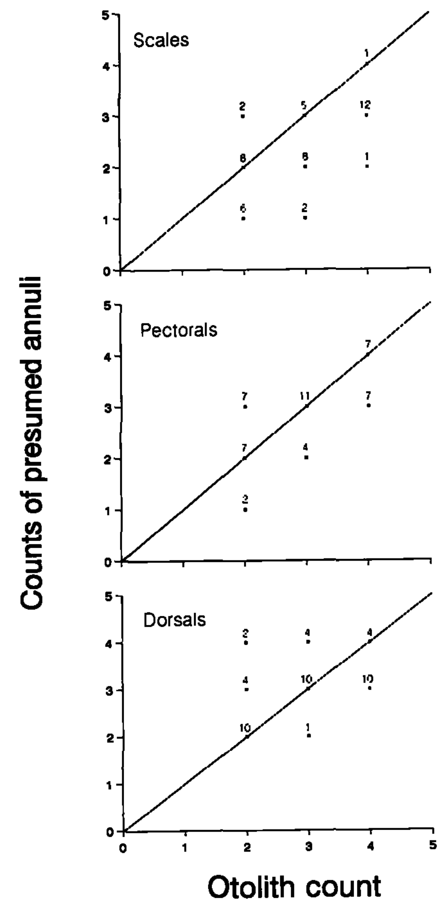

Figure 3
Counts of presumed annuli from weakfish, *Cynoscion regalis*, scales, pectorals, and dorsals compared with otoliths. The number of fish each point represents is indicated. The 45° line represents 100% agreement.

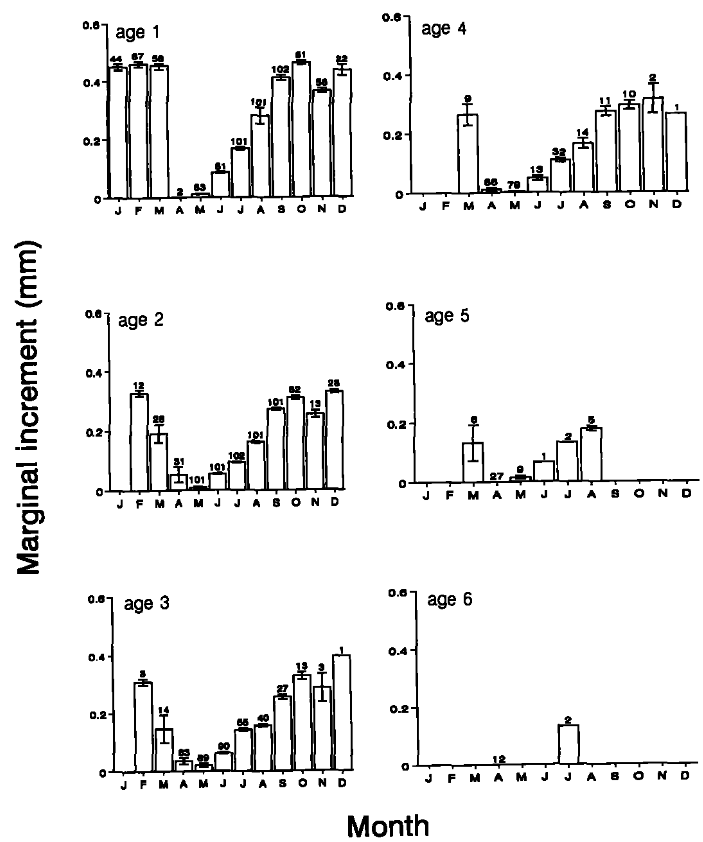

Figure 4
Mean monthly otolith marginal increments for weakfish, *Cynoscion regalis*, ages 1–6 from the Chesapeake Bay region, 1989–91. Vertical bars are ± one standard error. Numbers above the bars represent sample size.

The number of presumed annual marks on otolith sections agreed poorly with those on other hardparts (Fig. 3). Scale and otolith readings agreed only 27% of the time (Table 1) and scales consistently had one less mark than otoliths (26 out of 45). Pectoral and dorsal rays showed better agreement with otoliths than with scales, 49% and 46% respectively.

Validation of the otolith method

Opaque bands are laid down on otoliths once a year in the spring. Mean monthly marginal increment plots for ages 1–6 showed only one trough during the year, indicating only one opaque band was formed per year (Fig. 4). A few fish began to lay down annuli in March, as shown by the decrease in mean marginal increment and a relatively high variation in marginal increment size. However, lowest marginal increment values occurred in April and May, indicating most fish formed annuli during these months. Greatest otolith growth occurred during the months of June, July, August, and September, as demonstrated by the step-wise increase in mean marginal increments. By October, mean marginal increments reached a fairly stable maximum, indicating little or no otolith growth. This maximum continued until the next March or April, when annuli were again laid down.

Because of the scarcity of older fish, it was not possible to validate conclusively fish older than age 5 by separate marginal increment plots. However, there was no evidence that the pattern of annulus formation changed within the weakfish lifespan. Annuli were consistently formed during March–May for fish of different sizes, sexes, and ages (1–6), and otoliths did not form more than one mark per year even though these ages represented various stages in the fish's life history. Additionally, of the 2,217 otoliths examined (ages 1–10), all those in the process of forming or which had just formed annuli were collected in March–May. Thus, we assumed for ages 1–10 that the otolith method provided accurate ages.

Comparison of scales and otoliths

Scales were consistently more difficult to read than otoliths, and confidence in scale readings was often low. Percent agreement within and between readers was fairly consistent for both hardparts. However, otoliths showed much higher agreement (98–100%), than did scales (78–80%) (Table 2). Although agreement between scales and otoliths was fairly high, 79%, agreement decreased with increasing age. Of 32 disagreements, only 6 differed by more than one year (Fig. 5). However, 4 of the 5 fish older than age 6 were underaged by scales and two of the oldest fish, age 10 and 8, were underaged by 3 years. Scales from older fish, if they showed more than 6 annuli, had marks which were severely crowded and fragmented even when viewed at higher magnification (Fig. 6A), whereas otoliths from these same fish showed clear annuli (Fig. 6B).

Although the number of fish underaged was small, their effect on estimating growth curves would be dramatic. Mean body size at age based on scales, although slightly curvilinear, showed no clear indication of an asymptote (Fig. 7A) and thus would not be appropriate for fitting a von Bertalanffy growth curve (Gallucci and Quinn, 1979). In contrast, mean body size at age based on otoliths showed the clear beginnings of an asymptote (Fig. 7B).

Although sex of the fish had no effect on the precision or repeatability of scale readings, it did affect accuracy. Agreement of scale ages among and between readers was quite similar when calculated separately by sex, ranging from 75 to 79.5%. However, agreement between scale and otolith ages, or accuracy, was significantly different for males and females (χ^2=6.25, n=154, P<0.05). Of the 32 discrepancies between scale and otolith ages, 26 of them were males. Even if the fish greater than age 6 are discounted, there is still a significant difference (χ^2=5.79, n=149, P<0.05).

Time of annulus formation is not the same for scales and otoliths. Both hardparts showed only one trough in their mean monthly marginal increments (Fig. 8). However, otoliths with annuli on their margins were collected only during a discrete time period, 1 April–1 June, while scales in the process of forming annuli were collected from mid-April to mid-August, although most scales formed annuli in August. The variable and extended time of scale annulus formation is represented by the shallow trough (Brothers, 1983) and the larger standard errors of the scale marginal increment plot, as compared with that of otoliths (Fig. 8).

Although total length on hardpart size relationships for both scales and otoliths showed linear trends (r^2=0.94 and 0.88 respectively, n=175, P=0.0001), the total length on otolith relationship showed seasonal variation. When a single age class (age 3) was marked by season of collection and plotted against the linear relationship predicted by the total sample (Fig. 9), all fish collected in April and May had smaller than predicted otolith radii, whereas fish collected in August and September had larger than predicted radii. Fish collected in June and July were intermediate, although most of their radii were also smaller than predicted. Scales from the same fish did not show similar seasonal trends.

Back-calculation equations of total length on hardpart size were calculated only for fish collected at the beginning of the growing season, in April and

Table 2

Percent agreement of weakfish, *Cynoscion regalis*, scale- and otolith-assigned ages within readers, between readers, and between hardparts.

Hardpart	Within reader 1	Within reader 2	Between readers	With otoliths
Scales	80	78	80	79
Otoliths	100	98	99	

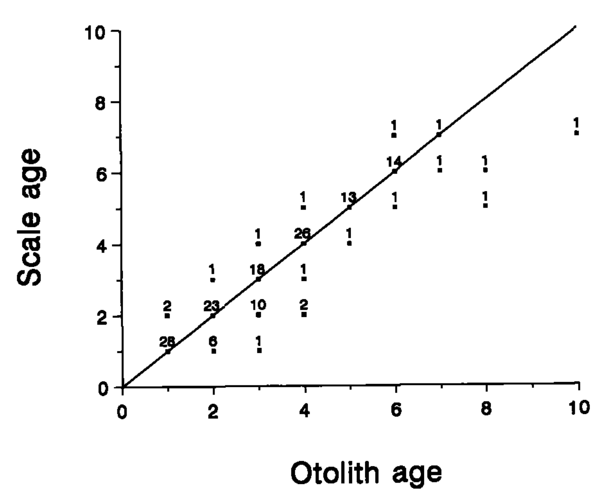

Figure 5

Weakfish, *Cynoscion regalis*, assigned ages from scales and otoliths. The number of fish each point represents is indicated. The 45° line represents 100% agreement.

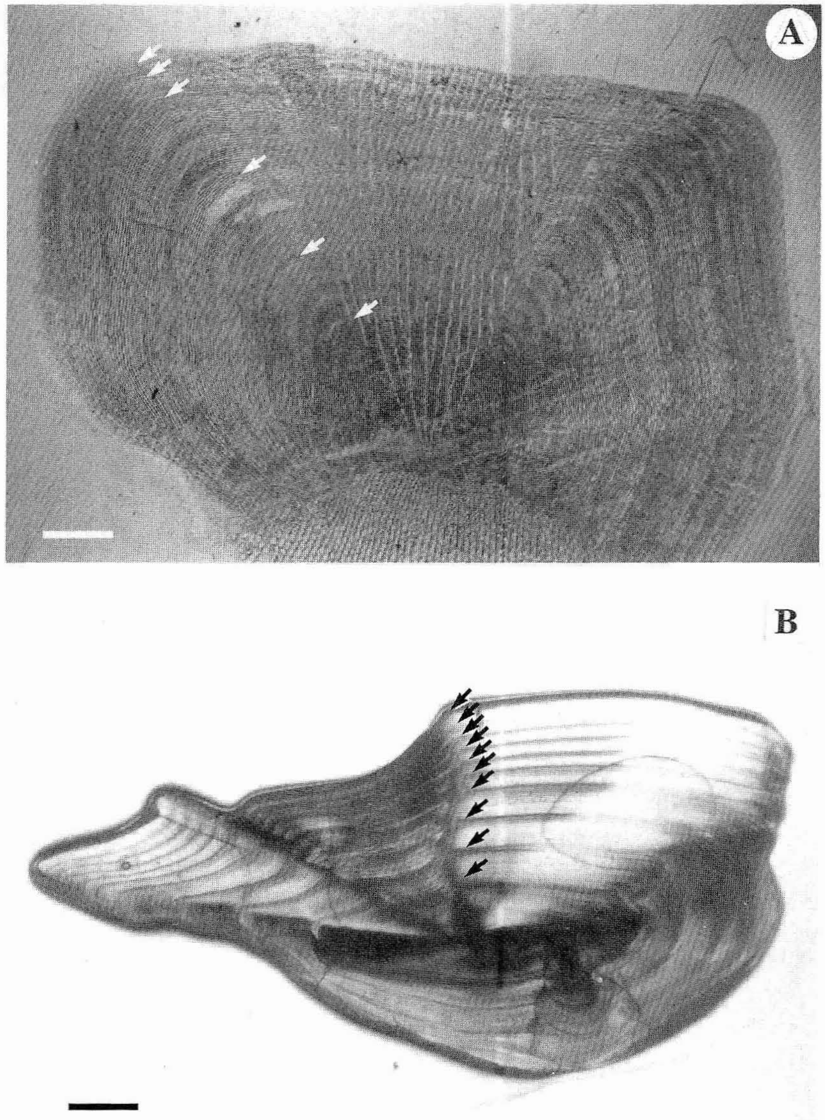

Figure 6

The scale impression (**A**) and sectioned otolith (**B**), as seen in transmitted light, from a male, 10-year-old weakfish, *Cynoscion regalis*, TL=845 mm, collected in mid-May. Arrows indicate marks counted as annuli.

May, to remove seasonal effects. Although linear regressions were significant for scales (r^2=0.95, P=0.0001) and otoliths (r^2=0.92, P=0.0001), a quadratic term improved the model fit and was significant (P=0.0003 scales, P=0.0001 otoliths) (Fig. 10). Equations were

For scales:

$$TL = -151.6 + 160.2\ SR - 5.4\ SR^2\ (r^2=0.96,\ n=88,\ P=0.0001);$$

For otoliths:

$$TL = -220.9 + 543.1\ OR - 66.9\ OR^2\ (r^2=0.94,\ n=88,\ P=0.0001).$$

The pattern of mean annual growth increments differed between scales and otoliths. Both scales and otoliths showed their largest growth increment from the focus to the first annulus (Fig. 11). However, once fish had reached age 1, the largest otolith annual growth increment occurred between the first and

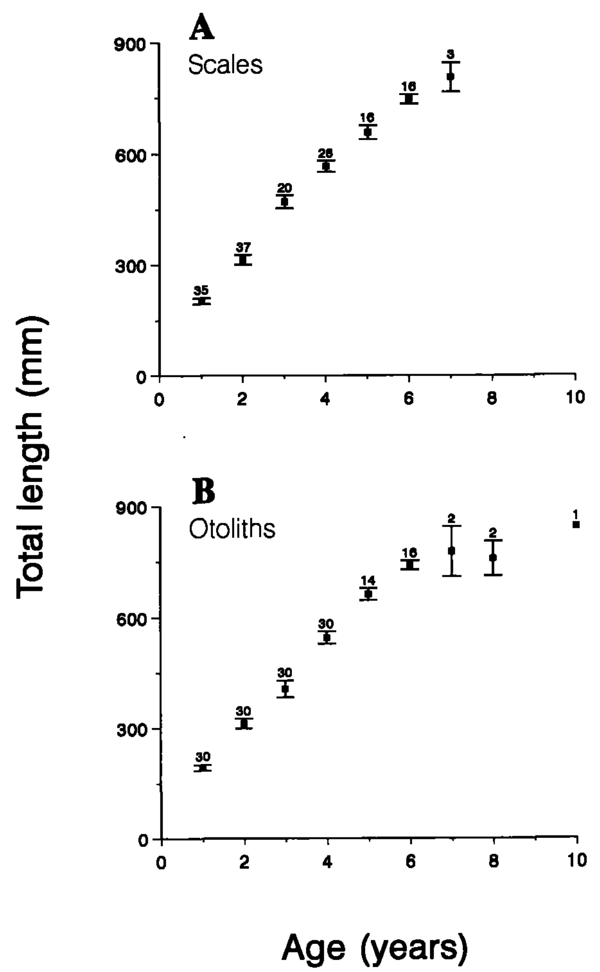

Figure 7

Mean weakfish, *Cynoscion regalis*, size at age: (**A**) based on scales and (**B**) based on otoliths. Vertical bars are ± one standard error. Numbers above the bars represent sample size.

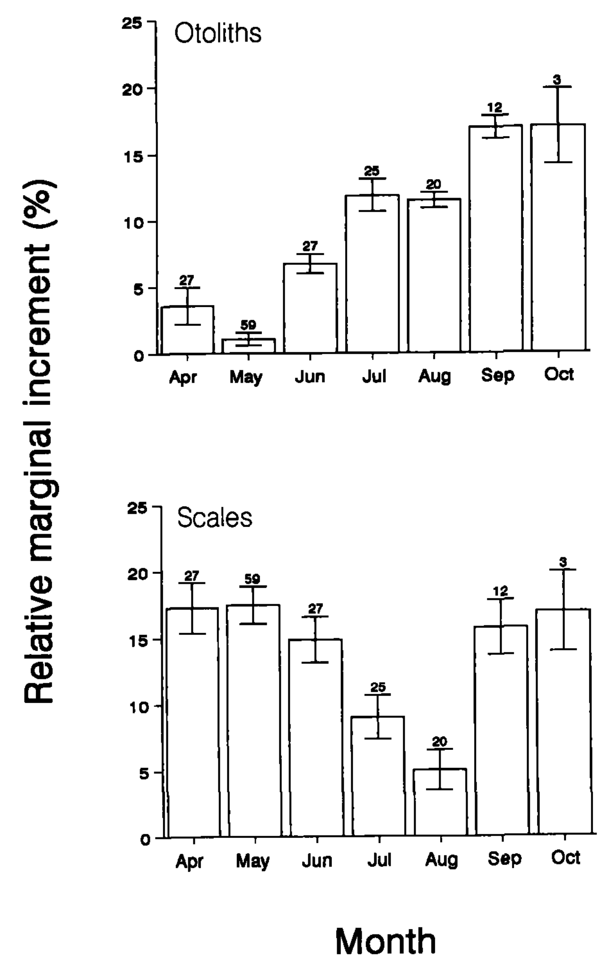

Figure 8

Mean monthly relative increments for weakfish, *Cynoscion regalis*, scales and otoliths. Vertical bars are ± one standard error. Numbers above the bars represent sample sizes.

second annuli, whereas scales had a very small increment between these annuli. The largest scale growth increment after age 1 was between annuli 3 and 4. Neither hardpart showed a consistently decreasing mean annual growth increment as age increased. Although this assumption is often included in scale-reading criteria, it would be inappropriate for weakfish.

Back-calculated mean body sizes at age were larger for scales than for otoliths (Table 3). In part, this discrepancy may reflect different times of annulus formation: back-calculated lengths from scales, in general, estimate sizes in August, whereas back-calculated lengths from otoliths estimate sizes in April and May. Also, at older ages, back-calculated body sizes at age based on scales would be expected to be larger because of the underageing of older fish by scales.

Both scales and otoliths showed smaller back-calculated mean body size at age 1 than observed. At later ages, back-calculated TL's from scales were larger than observed, while back-calculated TL's from otoliths showed no consistent trend (Table 3). The cause of the smaller back-calculated TL's at age 1, however, did not appear related to Lee's phenomenon, as there was no consistent trend of smaller age-1 annular radii at older ages at capture (Tables 4 and 5). In fact, the largest mean SAR and OAR at age 1 came from 5-year-old fish. However, age-1 OAR's from the oldest fish in the study (>age 6, n=5) were distinctly smaller than those observed in younger fish.

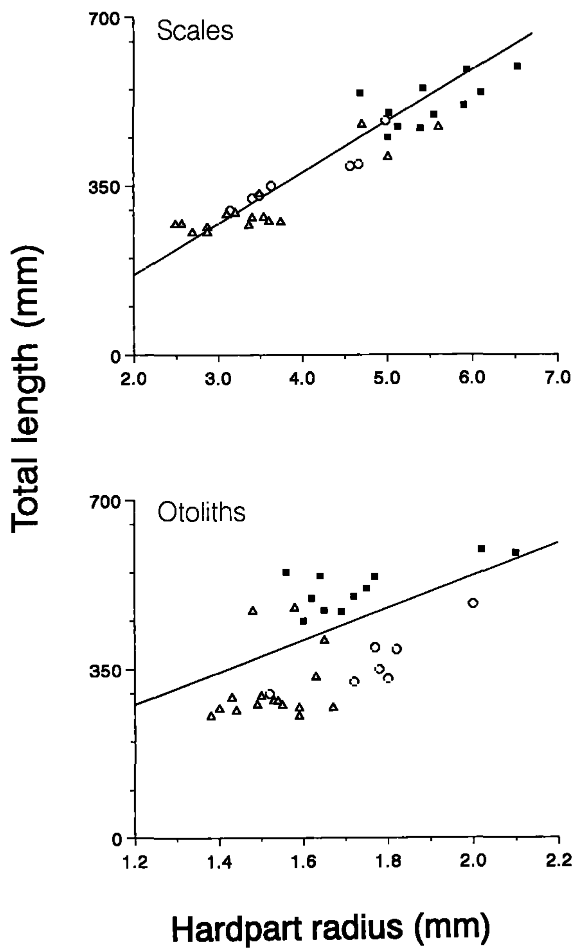

Figure 9

Total length plotted on hardpart size for age-3 weakfish, *Cynoscion regalis*, n=35. Lines represent the linear total length to hardpart regressions calculated from all fish, n=175. Fish are marked by season of collection: open circles=April–May; shaded triangles=June–July; and black squares=August–September.

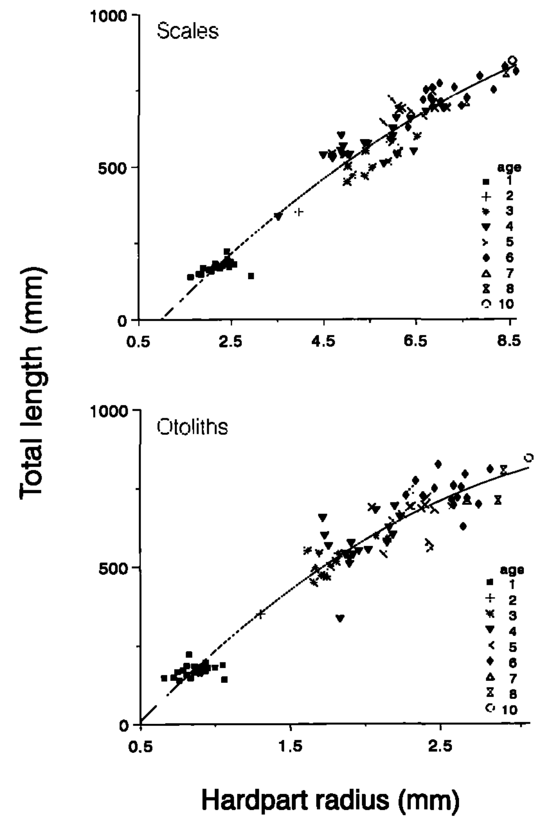

Figure 10

Weakfish, *Cynoscion regalis*, total length on hardpart radius regression used for back calculation. Based on fish collected in April and May, n=88.

Discussion

Our results indicate that transverse otolith sections are the best method to age weakfish. Sectioned otoliths were characterized by thin opaque bands, considered annuli, interspersed with wider translucent zones. This pattern is similar to other sciaenids, such as spotted seatrout, *Cynoscion nebulosus* (Maceina et al., 1987), Atlantic croaker, *Micropogonias undulatus* (Barbieri et al., 1994), red drum, *Sciaenops ocellatus* (Murphy and Taylor, 1991), and black drum, *Pogonias cromis* (Beckman et al., 1990). This pattern should not be confused with the more

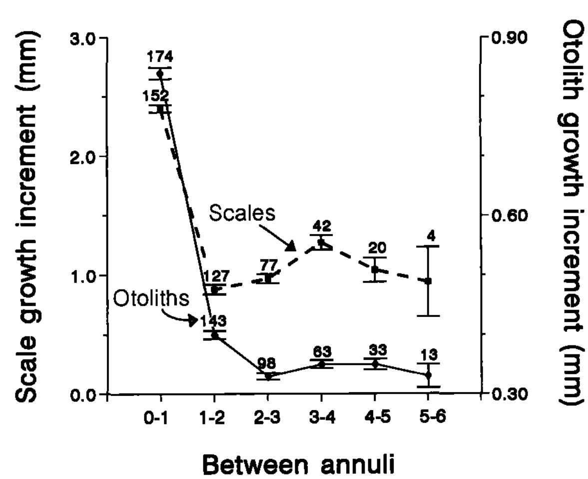

Figure 11

Mean annual growth increments of weakfish, *Cynoscion regalis*, scales and otoliths. Vertical bars are ± one standard error. Numbers above the bars represent sample sizes.

common otolith pattern found in many temperate fish of thin translucent zones, which are considered annuli, interspersed with wide opaque zones (Hyndes et al., 1992).

Table 3

Mean back-calculated weakfish, *Cynoscion regalis*, total lengths (mm) at age based on scales and otoliths, calculated from a quadratic body to hardpart regression and observed mean total length at time of annulus formation. Sample size is in parentheses.

Age	Scales	Observed Jul/Aug	Otoliths	Observed April/May
1	196 (152)	240 (7)	162 (174)	172 (22)
2	305 (127)	296 (25)	297 (144)	260 (2)
3	422 (77)	377 (8)	421 (99)	532 (12)
4	564 (42)	514 (5)	552 (64)	566 (18)
5	682 (20)		660 (34)	663 (14)
6	733 (4)		711 (14)	741 (16)
7			750 (5)	710 (1)
8			748 (2)	759 (2)
10				845 (1)

Table 4

Mean scale annular radii (SAR) for each scale age of weakfish, *Cynoscion regalis*.

		Scale annulus					
Age	*n*	1	2	3	4	5	6
1	12	2.59					
2	52	2.31	3.20				
3	24	2.40	3.42	4.14			
4	29	2.38	3.27	4.27	5.56		
5	16	2.65	3.44	4.31	5.43	7.15	
6	16	2.38	3.25	4.30	5.58	6.64	7.00
7	3	2.11	3.09	3.92	5.65	6.69	7.37

Table 5

Mean otolith annular radii (OAR) for each otolith age of weakfish, *Cynoscion regalis*.

		Otolith annulus							
Age	*n*	1	2	3	4	5	6	7	8
1	29	0.83							
2	45	0.85	1.27						
3	35	0.82	1.21	1.56					
4	30	0.82	1.20	1.53	1.91				
5	14	0.88	1.25	1.58	1.91	2.28			
6	16	0.86	1.22	1.54	1.88	2.21	2.52		
7	2	0.80	1.18	1.47	1.79	2.16	2.47	2.79	
8	2	0.77	1.20	1.56	1.90	2.22	2.47	2.65	2.85
10	1	0.67	1.11	1.52	1.94	2.15	2.32	2.49	2.67

Sectioned otoliths were consistently clear and easy to read, as shown by the high precision of repeated age readings. Although it was possible only to validate ages 1–5 by separate marginal increment plots, otolith annuli in all ages examined (1–10) were laid down once a year during a discrete time period (April–May). The constancy of annulus deposition at older ages, the lack of severely crowded annuli in older fish, and the similarity between weakfish otoliths and other sciaenid otoliths that have been validated at older ages (Beckman et al., 1990, Murphy and Taylor, 1991; Barbieri et al., 1994) suggest that otoliths are a reliable ageing technique for weakfish, although older ages must still be validated.

In contrast, we found the scale method of ageing weakfish to be imprecise and apparently inaccurate at older ages. We found that scales form annuli over an extended period, April–August, similar to the results of past studies (Perlmutter et al., 1956; Massmann, 1963b). This protracted period of annulus formation made it difficult to assign ages to fish taken in midsummer with moderate growth on the scale margin, as noted by Massmann (1963b). For example, a fish taken in July with a medium marginal increment on its scale could have formed its annuli in early April and have grown since then, or it could have increased its growth increment before forming an annulus in August. Thus, assigning an age to these fish is purely subjective and can lead to ageing errors ± one year, which may explain most of the discrepancies between otolith and scale ages.

The long period of annulus formation on scales and the severe crowding of annuli at older ages make it difficult to validate scales by the marginal increment method—as Perlmutter et al. (1956) and Shepherd and Grimes (1983) attempted for pooled age data. Because scale annuli form over a protracted period, the trough in the marginal increment plot is shallow and the range of marginal growth during other months is large. Additionally, validation by the marginal increment method is not appropriate if the hardpart shows severe crowding of annuli at older ages, as we found with scales, and has been previously reported (Shepherd, 1988). Shepherd (1988) described annuli in fish older than age 6 or 7 as being crowded and very difficult to detect, which could lead to marginal increments being measured from the last distinguishable annulus to the edge, rather than from the last real annulus to the edge. This error would inflate marginal increment estimates and there would be no way to detect underaged, older fish in marginal increment plots.

The scale method appears to underage older weakfish. Assuming otolith ages were valid, 4 of the 5 fish in this study older than age 6 were underaged by scales. Although 4 out of 155 fish may seem insignificant, the importance of correctly ageing these fish cannot be judged only by the number of discrepancies. These fish represent the beginning of an asymptote in growth and fish in the asymptotic range are often rare in highly exploited stocks. Obtaining and correctly ageing a few weakfish in this range is critical to correctly estimating the parameters of the von Bertalanffy growth curve.

Annulus formation on weakfish otoliths and scales shows different patterns. The formation of otolith annuli over a discrete time period suggests it may be caused by environmental variables. The most commonly suggested environmental influences on annulus formation are temperature, salinity, food, and light (Simkiss, 1974). Weakfish form annuli on their otoliths in April and May, when they migrate from offshore winter grounds to estuarine feeding and spawning grounds. Thus, annulus formation may be linked to their migration into a different environment.

Weakfish scales, in contrast, have a more variable time of annulus formation suggesting a cause other than general environmental conditions. Scales may undergo resorption whereas otoliths do not (Simkiss, 1974), and spawning has been linked to scale resorption with a consequent scale mark in salmon and trout (Crichton, 1935). Spawning may also be linked to formation of annuli on weakfish scales (Merriner, 1973). Weakfish mature at age 1 (Merriner, 1976; Shepherd and Grimes, 1984) and are multiple spawners with a protracted spawning period from May through August (Lowerre-Barbieri[2]). However, individual spawning periods are asynchronous and vary greatly, especially in time of termination. Spawning activity and annulus formation may be linked in two ways: 1) annuli could form on scales early in the spawning season when resources are shifted towards production of reproductive materials—especially the yolking of oocytes, or 2) annuli might form near the end of the season, owing to the cumulative drain of protracted spawning, causing a cessation in growth and thus an annulus. A connection between scale annulus formation and spawning in weakfish would explain the high level of variation in time of annulus formation and the higher accuracy of ages based on scales taken from females, because females usually invest more energy in reproduction. It might also explain the small growth increment between annuli 1 and 2 if one-year-old weakfish begin spawning later in the season than older fish, owing to a threshold size necessary to reach maturity.

Our results indicate both scales and otoliths present problems for back-calculation of weakfish. Although scales showed a strong relationship between body and hardpart size and no seasonal differences in growth, their long and variable time of annulus formation may cause considerable error (Smith, 1983). It is impossible to determine if a fish formed its annuli at the same time each year. Because annuli can form from April to August, increments may represent 8–16 months of growth rather than approximately one year of growth. Additionally, scale annuli are more difficult to distinguish than otolith annuli, making SAR's difficult to measure and somewhat subjective. However, otoliths show seasonal change in the body to hardpart relationship, making a season-specific back-calculation equation, such as we developed, inappropriate for fish collected outside of that season. Additionally, comparisons between back-calculated and observed sizes at age were complicated by the weakfish migrational pattern, since weakfish age ranges in the Chesapeake Bay vary seasonally—older fish are present only in spring and only occasionally in fall (Joseph, 1972).

There was no clear evidence of Lee's phenomenon, as older fish did not consistently show smaller hardpart size at younger ages. The five oldest fish did, however, demonstrate considerably smaller OAR's at age 1 than did their younger counterparts. Nevertheless, these same fish did not demonstrate consistently smaller OAR's at consecutive ages than did younger fish. Thus, the smaller OAR's at age 1, rather than demonstrating Lee's phenomenon, may simply reflect when most fish of those year classes were born, i.e. fish born early in the spawning season would have larger OAR's at age 1 because they had more time to grow before winter, than did fish born later in the season.

Previous criticism of back-calculation has focused mainly on the body size to hardpart relationship and its calculation (Campana, 1990; Casselman, 1990; Francis, 1990; Ricker, 1992). However, the validity of back-calculation also depends on the constancy, clarity, and pattern of hardpart growth increments. The different growth increment patterns we found between scales and otoliths demonstrate the need to understand hardpart growth better, how it relates to somatic growth and what causes annulus formation on different hardparts.

Future studies of weakfish age and growth should be based on sectioned otoliths because scales appear inaccurate once growth becomes asymptotic. This common failing of the scale method has been reported for many species (Beamish and McFarlane, 1987). It

[2] Lowerre-Barbieri, S. K. 1993. Reproductive biology of weakfish, *Cynoscion regalis*, in the Chesapeake Bay region. School of Marine Science, VIMS, College of William and Mary, unpubl. manuscr.

can result in underestimates of longevity, overestimates of mortality, inaccurate growth calculations, and improper modelling and management decisions (Beamish and McFarlane, 1983). Similarly, current estimates of weakfish growth, longevity, and mortality may need to be reevaluated, as suggested by our findings that scales underage older fish and have crowded annuli past age 6. The need for this reevaluation is underscored by the recording of a 17-year-old, as aged by otoliths, which was previously aged as a 7-year-old by scales (Lowerre-Barbieri[3]).

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