Differences between the sagitta, lapillus, and asteriscus in estimating age and growth in juvenile red drum, *Sciaenops ocellatus*

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Abstract.—Most red drum, *Sciaenops ocellatus*, age and growth research has utilized sagittal otoliths. We evaluated the other otoliths, the lapilli and asterisci, as well as the sagittae, as ageing structures and length-at-age estimators in hatchery-reared and wild juveniles (<50 mm SL). Our otolith mounting and sectioning protocol for preparing sagittal sections required significantly less processing time with no loss in accuracy than the more traditional transverse sectioning reported in the literature. Increments in asterisci were clearly visible from the primordium to the otolith margin, whereas the nuclear region of sagittae and lapilli were more opaque and prevented detection of all rings. Asterisci were not present at hatching, but on average form six days later, therefore the addition of a constant (6 days) to the ring counts of asterisci resulted in this otolith providing more accurate ages than sagittae or lapilli. According to coefficients of determination (r^2) generated from the relation between fish length and otolith diameter, the sagitta, asteriscus, and lapillus predict fish length in descending order. However, because inner rings on sagittae were usually undetectable, lengths at ages cannot accurately be predicted. All rings are observable in the asteriscus; thus, by using the 6-day adjustment factor accurate length-at-age prediction is possible.

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The red drum, *Sciaenops ocellatus*, is a recreationally and commercially important marine fish ranging throughout the Gulf of Mexico and along the Atlantic seaboard to Massachusetts. An estuarine-dependent species, red drum spawn offshore, and juveniles move to estuarine nursery areas. After reaching sexual maturity at about age 5 (Mercer, 1984), these fish migrate offshore, where they aggregate into large schools. Concern for the declining status of the resource has prompted many Gulf of Mexico and Atlantic states to implement research to provide a sound scientific basis for management. This research includes aspects of early life history important to recruitment processes (Holt et al., 1983), age and growth (Peters and McMichael, 1987; Comyns et al., 1989), artificial propagation (Arnold, 1988), behavior (Fuiman and Ottey, 1993), and spawning stock abundance (Comyns et al., 1991).

An overwhelming percentage of age and growth investigations have utilized the sagittae because they are the largest of the three otoliths and therefore the easiest to extract and examine (Irie, 1960; Campana and Neilson, 1985). Since Panella (1971) first identified daily growth increments in sagittal otoliths, the formation of daily rings has been confirmed in many species (Jones, 1986).

References to the lapillus are uncommon in the literature. Bailey and Stehr (1988) reported use of the lapillus to age larval walleye pollock, *Theragra chalcogramma* (Pallas), younger than 20 days because it was larger than the sagitta up to that age. Brothers and McFarland (1981) used lapilli to age juvenile French grunts, *Haemulon flavolineatum*. References to the use of the asteriscus in ageing are unknown.

Previous ages for red drum larvae (Comyns et al., 1989) and juveniles (Peters and McMichael, 1987) were derived from sagittae. Peters and McMichael (1987) found that the inner ring structure was usually unclear in juveniles. They used ten larval otoliths to measure the distance from the primordium to the tenth ring, then began juvenile ring counts with the eleventh ring, 56 µm

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from the primordium (the mean distance to the tenth ring from the ten larval otoliths). The juvenile otoliths were transversely sectioned and glycerin-cleared. This method was verified by examination of the innermost rings from exceptionally clear juvenile otoliths.

We examined sagittae, lapilli, and asterisci to determine the best ageing structure for juvenile red drum by comparing ring counts for each otolith with known ages in hatchery-reared fish. We also evaluated the relation between fish length and otolith diameter for use in backcalculating lengths at age from each otolith. We report the size at which otoliths are formed to evaluate possible biases in age estimations.

Materials and methods

Wild-caught and known-age (laboratory-reared) juvenile red drum were preserved in 70% ethanol. Standard lengths of fish were measured to the nearest 0.1 mm prior to the removal of otoliths. We made no attempt to measure shrinkage due to preservation or to adjust our measurements accordingly. Otoliths were extracted between crossed polaroids by using a dissecting microscope at 6–12× magnification and transmitted light. This procedure exploited the birefringence of the crystalline structure of otoliths and greatly aided otolith location and removal. Extraneous tissue was removed, and the otoliths were mounted concave side up on glass slides. Two mounting media were used, thermoplastic cement and a polymer mounting medium. The thermoplastic cement allowed easier otolith manipulation and the polymer provided a less brittle and more transparent mount. Otoliths were ground in the sagittal plane with 600-grit carborundum paper and polished with 0.9-µm aluminum oxide sheets prior to examination under a compound microscope. Otoliths mounted with thermoplastic cement were ground to the primordium, flipped convex side up, and again ground to the primordium. Polymer mounted otoliths were ground to the primordium from the concave side. The polished otoliths were illuminated with plane-polarized transmitted light to optimize ring resolution. Ring counts were determined at 125–250× for sagittae and at 250–500× for lapilli and asterisci. To avoid bias from sequentially ageing all three otoliths from the same fish, all sagittae were aged first, followed by all lapilli and all asterisci. After ageing, mean ring counts, standard deviations, and coefficients of variation were calculated.

Otolith diameters (µm) were measured with a digital image analysis system (Optimas ver 3.01, Bioscan, Edmunds, WA). Otolith diameter was defined as a chord from the anterior rostrum to the posterior edge, passing through the primordium. The relationship between standard length and otolith diameter was examined by plotting otolith diameter on standard length and calculating the regression.

We examined larvae and juveniles to determine the size at which otoliths were formed. Larvae were translucent under transmitted polarized light, so otoliths were observable without dissection in fish as small as 4.0 mm SL. To precisely determine fish size at asteriscus formation, five larvae between 2.7 and 3.5 mm SL were dissected and the disrupted otic capsules mounted on glass slides with polymer mounting medium and examined microscopically.

Results

Wild-caught fish ranged from 15 to 50 mm SL, and laboratory-reared fish ranged from 18 to 25 mm SL. Fifty laboratory-reared and 70 wild-caught juveniles were processed; six otoliths were removed from each fish. The sagittae were used as landmarks to locate the smaller lapilli and asterisci. The lapilli were anterior and distal to the sagittae, and the asterisci were posterior and proximal to the sagittae. The asterisci were often found attached to the saccular tissue removed with the sagittae. To determine the size at otolith formation, we examined 35 wild-caught red drum larvae ranging from 1.3 to 7.0 mm SL. Sagittae and lapilli were present in all larvae examined. Asterisci were found in all dissected fish ≥3.0 mm SL (n=5) and not detected in fish smaller than 2.8 mm SL (n=3). In specimens that were not dissected, asterisci were first observed in larvae 3.8 mm SL and were present in all larvae larger than 4.0 mm SL.

Considerable differences were found in the general size and shape of the otoliths (Fig. 1). All otoliths were spherical or slightly ovoid in larvae ≤5 mm SL. Sagittae in larvae ≥5 mm SL began to develop a rostral process, and sagittae of 10-mm larvae were oval, laterally compressed, and had developed a prominent rostrum. The general shape of sagittae did not change in fish 10–50 mm SL. Lapilli followed the same initial development, but the posterior margin of these otoliths became scalloped when larvae were approximately 15 mm SL owing to the formation of numerous accessory primordia. Initially asterisci were also spherical, but the axis of growth changed, with subsequent development resulting in a kidney-shaped appearance by 15 mm SL. Accessory primordia were seldom observed in sagittae and were not observed in asterisci. Lapilli and asterisci were similar in size; however, the diameter of sagittae was approximately three times larger and grew approximately five times faster than asterisci and lapilli (Fig. 2).

Diameters of asterisci and lapilli were well correlated with standard length, but the strongest relation between otolith diameter and standard length was observed in sagittae (Fig. 2). Anterior rostra and

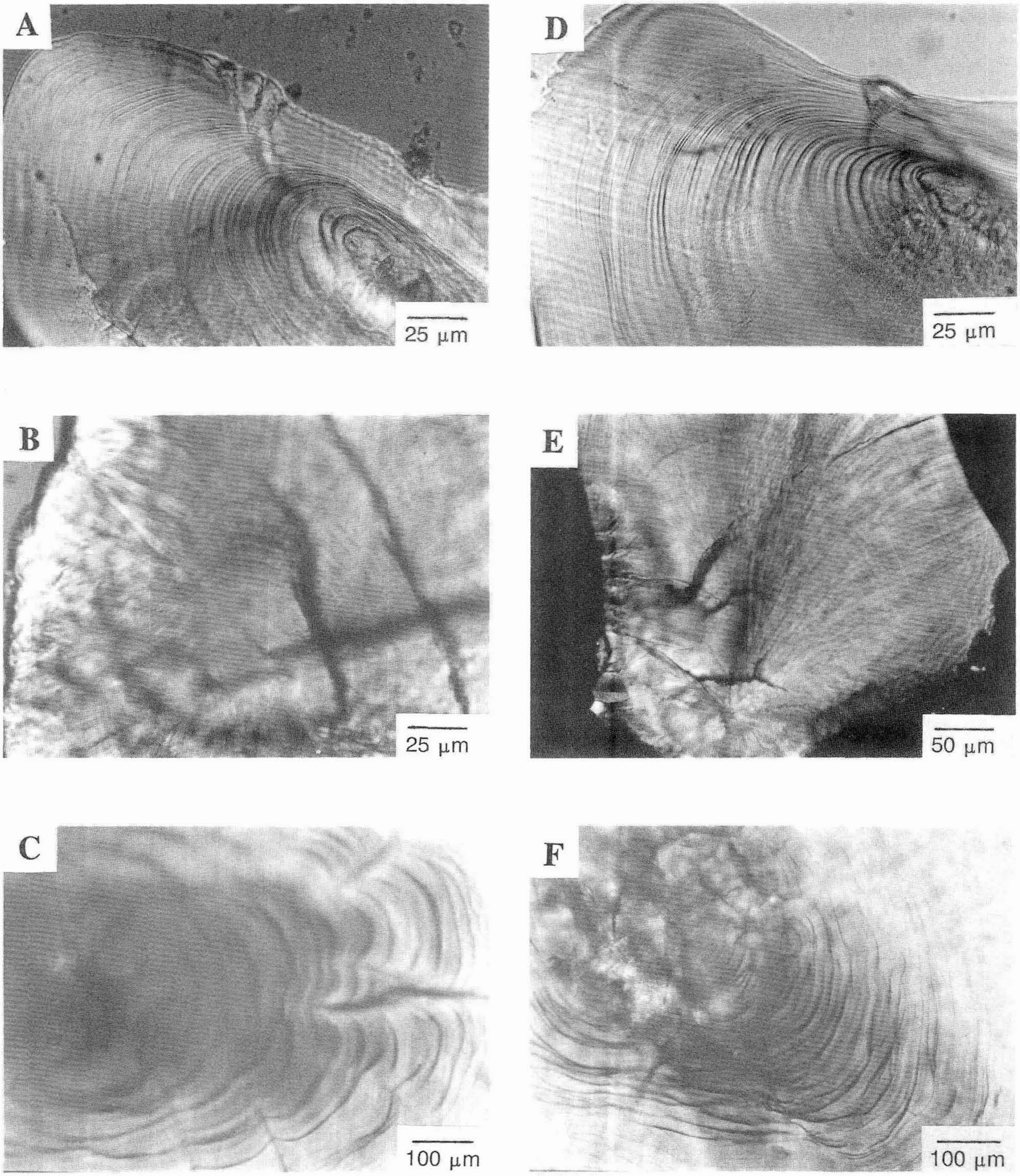

Figure 1

Red drum, *Sciaenops ocellatus*, otoliths: (**A**) hatchery asteriscus 500×; (**B**) hatchery lapillus 500×; (**C**) hatchery sagitta 125×; (**D**) wild asteriscus 500×; (**E**) wild lapillus 250×; (**F**) wild sagitta 125×.

otolith primordia were well defined in sagittae, which, along with a uniform axis of growth, resulted in a more consistent axis of measurement. The lapillus and asteriscus were more circular in shape than the sagitta and had a less well-defined anterior rostrum. The lapillus also formed accessory primordia, which were reflected in an irregular perimeter. Although accessory primordia were not observed in asterisci, the axis of growth shifted with development, resulting in diameter measurements through the primordia that did not necessarily reflect the maximum otolith diameter. These features resulted in greater variability of diameter measurements for asterisci and lapilli than for sagittae.

The accuracy and precision of age estimates varied among otoliths. Fish used to estimate daily ages were collected 46 days after hatching. Although increments were present in all three otoliths, the definition and resolution of the rings differed, especially near the primordia. The mean ring counts, standard deviations, and coefficients of variation are presented in Table 1. All increment widths exceeded the limit of resolution for light microscopy by more than an order of magnitude (Jones and Brothers, 1987; David and Paul, 1989). All ring counts substantially underestimated the age of the 46-day-old hatchery-reared fish. The asteriscus underestimated the true age by 6 days, while sagittae and lapilli underestimated age by 21 days and 25 days, respectively. To obtain realistic age estimates using asterisci, ring counts were adjusted because that otolith is not formed at hatching, but age estimates from ring counts in lapilli and sagittae were erroneously low because all rings could not be observed and counted because of poor contrast in the nuclear region. In addition to providing the most accurate age, coefficients of variation indicated that the asteriscus was also the most precise indicator of true age.

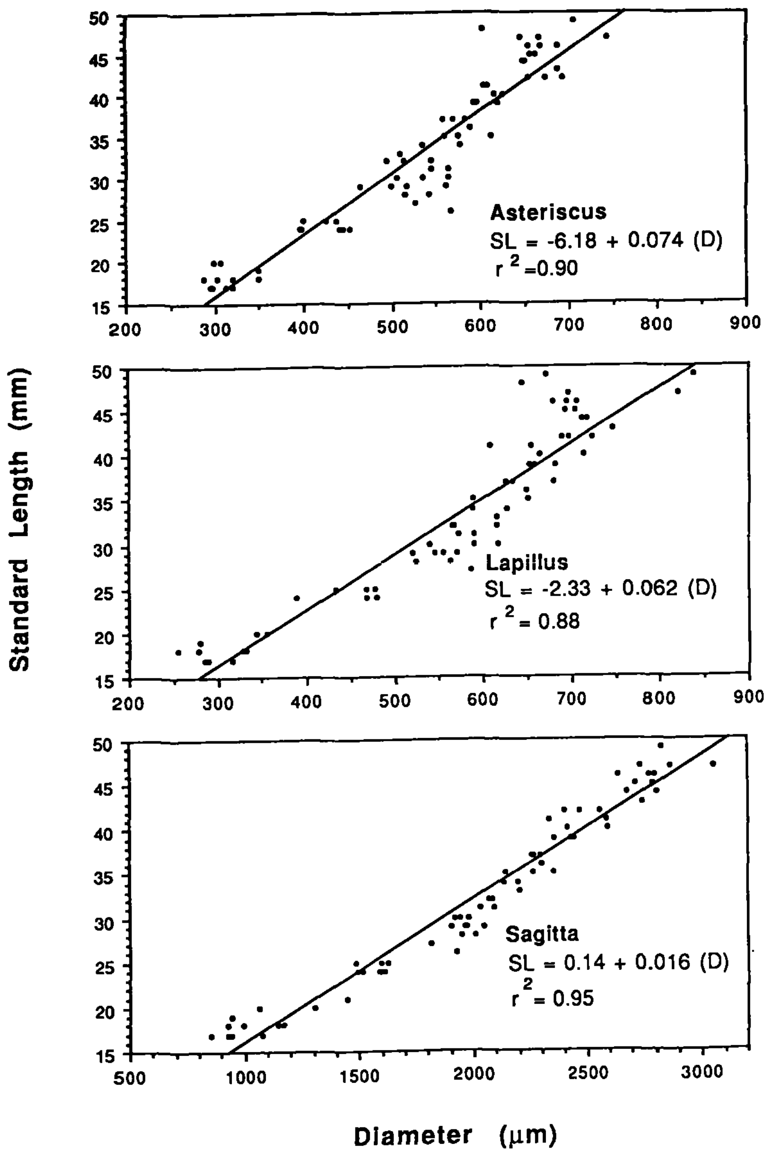

Figure 2

Fish length and otolith diameter relationships for each otolith type of red drum, *Sciaenops ocellatus*. Otolith diameter (µm) plotted on standard length (mm) with linear regression lines overlaid (*n*=70).

Discussion

Shrinkage of larvae due to preservation has been found to be significant (Blaxter, 1971; Theilacker and Dorsey, 1980; Hay, 1982; Brothers et al., 1983; and Leak, 1986). We did not adjust length measurements for shrinkage due to preservation because most specimens were juveniles (15–50 mm SL), and because we immediately fixed specimens in ethanol which has been shown to minimize the problem of shrinkage (Radke, 1989).

Daily increment formation has been validated in sagittae by using laboratory-reared red drum that were up to 21 days posthatch (Peters and McMichael, 1987). The 6-day underestimate in age determined from the asteriscus of known-age (hatchery-reared) fish corresponded well with age at otolith formation (6–7 days)

Table 1

Comparison of ages derived from sagittal sections of each otolith type in known age (46 days), hatchery-reared red drum, *Sciaenops ocellatus*. Values are reported for mean age, standard deviation, and coefficient of variation.

Otolith	*N*	Mean age (d)	SD	CV
Asteriscus	50	39.7	3.2	8.0
Sagitta	50	25.0	4.6	18.6
Lapillus	50	21.0	5.5	26.3

determined by examination of the age series of larvae, indicating that rings were indeed formed daily, and all rings were visible. Therefore, we assumed that rings were also formed daily in the lapillus and that underestimation of age was due to the inability to observe rings of low contrast in the opaque nuclear area rather than to other possibilities that have been reported, i.e. nondaily ring formation due to poor growth in herring, *Clupea harengus*, and turbot, *Scophthalmus maximus* (Geffen, 1982), and to ring spacing below the resolution limit of light microscopy in striped bass, *Morone saxatilis*, under suboptimal feeding regimes (Jones and Brothers, 1987).

Peters and McMichael (1987) had difficulty distinguishing the innermost rings in sagittae of some juveniles, and they developed an ageing method that did not require counting these rings. This method utilized transverse sections of larval sagittae in which rings were clearly visible to determine the distance from the primordium to the tenth ring. Subsequent juvenile ring counts were initiated at this distance away from the primordium. We also encountered difficulty in detecting all the rings near primordia in sagittae (and lapilli as well) due to the opacity of the nuclear region of the otoliths, but all rings were usually clearly visible in asterisci. Peters and McMichael (1987) made relatively accurate ring counts beyond the tenth ring on sagittae of 21-day-old known-age fish. However, we were not able to duplicate their success using our technique, because our counts underestimated the true age of 46-day-old fish by 21 days with a coefficient of variation of 18.6%. Duplicating their glycerin soaking technique did not improve our ring detection capability. The principle difference between our method and that of Peters and McMichael (1987) was the sectioning plane; they used the transverse plane, whereas we used the sagittal plane.

Because the otoliths were birefringent, they were easily observed within larval fish when illuminated with transmitted light between crossed polaroids. Sagittae and lapilli were easily seen in fish as small as 1.3 mm SL, whereas asterisci were observed in all fish greater than 3.0 mm SL. Comyns et al. (1989) detected two rings in sagittae and lapilli of larvae 2 days after fertilization (1 day posthatch), demonstrating that sagittae and lapilli are present at hatching; according to their growth curve, fish 3.0 mm SL were approximately 6 days old. Whereas the size at hatch was relatively constant, growth varied considerably with temperature, but the variation was not significant until the fish attain 4.0 mm SL (Comyns et al., 1989). Changes in growth rates due to water temperature are therefore not likely to have a significant effect on our estimate of age at asteriscus formation. Others have reported asteriscus formation at similar ages, e.g. at age 6 days in the Japanese eel, *Anguilla japonica* (Umezawa et al., 1989).

The asteriscus provided the most accurate estimate of age for juvenile red drum because it underestimated true age by only 6 days compared with 21 days for. the sagitta and 25 days for the lapillus. The asteriscus ages were also the most precise, because the coefficient of variation was only 8.0% compared with 18.6 and 26.3% for the sagitta and lapillus respectively. Most of the variance in age estimates for all three otoliths was caused by the inability to resolve rings near the primordium and at the margin. Rings in the mid portion of asteriscus sections were consistent in shape, increment width, and clarity; a dominant, consistently identifiable, first ring was visible at the edge of the primordium.

The sagitta provided the next most accurate and precise estimates. Ages were underestimated by 21 days on average, and rings near the primordia were difficult to distinguish, resulting in a coefficient of variation more than a factor of two higher than that of the asteriscus. Because sagittae were present at hatching and early rings were detectable in smaller and younger fish (Peters and McMichael, 1987; Comyns et al., 1989), better accuracy and precision of ageing using sagittae may be possible with improvements in preparation and processing of the otolith. However, the Spurr mounting technique (Haake et al., 1982) used by Peters and McMichael (1987) and Comyns et al. (1989) can require several days for proper dehydration, curing, sectioning, and polishing whereas our polymer method required less than one hour to produce ground and polished slides from whole fish, thus allowing considerably more fish to be processed in comparable time periods.

Ages estimated from the lapillus were least accurate and precise because of poor clarity of rings near the primordia and because of the formation and fusion of numerous accessory primordia. This fusion of accessory primordia resulted in superimposition of rings near the margin and the presence of several planes of growth being visible in the same focal plane.

Although additional preparation may have increased resolution of rings near the primordia, poor ring resolution caused by accessory primordia would probably not have been improved.

The addition of a constant to asterisci ring counts (6 days) adjusted for the time lag between hatching and otolith formation and was not used to compensate for uncounted rings. The addition of constants to estimates of age did not affect the rank of the coefficients of variation.

All otolith diameters, especially sagittae, exhibited a strong correlation with fish lengths. This relation can be useful in backcalculating size at age. However, because the ring count did not accurately estimate age, accurate ages derived from sagittae can not be associated with back-calculated sizes. The relation between asteriscus diameter and fish length was not as strong as with sagittae, but the improvements in accuracy and precision in estimating age would increase the confidence in back-calculated sizes at age. However, because asterisci were not present at hatching, size-at-age information for fish <7 days old could not be backcalculated. Rings formed in the asteriscus were concentric and proportionally spaced throughout the otolith because no accessory primordia were formed, making it straightforward to measure radii or increment widths precisely regardless of the chosen axis of measurement.

We conclude that the asteriscus is the best structure to use in ageing young red drum >4.0 mm SL. Using our sagittal section technique with juveniles, we found that the asterisci clearly provided superior accuracy and precision in ageing. Previous efforts by Peters and McMichael (1987) to age juvenile red drum using transverse sections of sagittae provided reasonable age estimates; however, because they began counts a standard distance from the primordium to allow for uncounted rings (10), the accuracy and precision of resulting counts on 21-day-old fish cannot be known in the same sense that we estimated these statistics when all rings were counted on asterisci of 46-day-old fish. The advantage of using asterisci is that clear rings can be seen in sagittal sections, and grinding asterisci in the sagittal plane can be done relatively quickly. Furthermore, all rings were visible in the asteriscus, and only adjustment for age at formation was required to estimate true age.

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