Effect of type of otolith and preparation technique on age estimation of larval and juvenile spot (*Leiostomus xanthurus*)

Dariusz P. Fey

Sea Fisheries Institute
Dept. of Fisheries Oceanography and Marine Ecology
ul. Kollataja 1
81-332 Gdynia, Poland
E-mail address: dfey@mir.gdynia.pl

Gretchen E. Bath Martin

James A. Morris

Jonathan A. Hare

NOAA National Ocean Service
Center for Coastal Fisheries and Habitat Research
101 Pivers Island Road
Beaufort, North Carolina 28516-9722

Otoliths of larval and juvenile fish provide a record of age, size, growth, and development (Campana and Neilson, 1985; Thorrold and Hare, 2002). However, determining the time of first increment formation in otoliths (Campana, 2001) and assessing the accuracy (deviation from real age) and precision (repeatability of increment counts from the same otolith) of increment counts are prerequisites for using otoliths to study the life history of fish (Campana and Moksness, 1991). For most fish species, first increment deposition occurs either at hatching, a day after hatching, or after first feeding and yolksac absorption (Jones, 1986; Thorrold and Hare, 2002). Increment deposition before hatching also occurs (Barkmann and Beck, 1976; Radtke and Dean, 1982). If first increment deposition does not occur at hatching, the standard procedure is to add a predetermined number to increment counts to estimate fish age (Campana and Neilson, 1985).

Accuracy and precision of increment counts is in part determined by the increment formation rate, which has been reviewed elsewhere (Campana and Neilson, 1985; Jones, 1986; Geffen, 1987), and by the type of otolith (asteriscus, sagitta, or lapillus) and the preparation technique used for aging. In most age and growth studies of larval and juvenile fish, the sagitta, the largest of the three otoliths, has been used (Campana and Neilson, 1985), but there are many examples of fish species that can be aged accurately by using the lapillus (e.g., Hoff et al., 1997; Bestgen and Bundy, 1998; Escot and Granado-Lorencio, 1998; Morioka and Machinandiarena, 2001). Although infrequently used, the asteriscus has provided age information with similar or even better precision and accuracy than the sagitta and lapillus (David et al., 1994). However, the microstructure of asterisci is usually not as clear as that of sagittae or lapilli, and the extraction of asterisci is relatively time consuming and laborious (Campana and Neilson, 1985; Neilson and Geen, 1985). As for otolith preparation, two general techniques are common: 1) polishing of one or both sides of a sectioned otolith in transverse view, and 2) polishing of one side of the whole sagitta (Secor et al., 1992). Sagittae and lapilli provide the same accuracy and precision for age estimation; however, lapilli may be easier to process for age determination and may not require processing at all (e.g., Ichimaru and Katsunori, 1995).

Spot (*Leiostomus xanthurus*) is an important fishery species along the southeast coast of the United States (Mercer, 1987) and is a dominant species in coastal ecosystems owing to its abundance (Walter and Austin, 2003). Studies of spot have illuminated processes that affect the abundance of estuarine-dependent species (Warlen and Chester, 1985; Flores-Coto and Warlen, 1993; Ross, 2003). Further, spot has been used as an experimental organism for examining larval ecology (Govoni et al., 1985; Govoni and Hoss, 2001) and otolith chemistry (Bath Martin et al., 2000, 2004; Bath-Martin and Thorrold, 2005). Although spot has been widely studied and is an important ecological and fishery species, basic information necessary for otolith analyses is not available.

Our goal was to provide a foundation for the use of otolith increment counts in examining the ecology of larval and juvenile spot. Our specific objectives were 1) to determine the timing of first-increment formation of spot (*Leiostomus xanthurus*) and 2) to assess the accuracy and precision of age estimates from increment counts made with different combinations otoliths and preparation techniques. Specifically, four combinations of otoliths (sagittae and lapilli) and preparation techniques were compared: 1) a transverse section of the sagitta (polished on one side TSS-1); 2) a transverse section of the sagitta (polished on two sides TSS-2); 3) a whole sagitta (polished on one side WS-1); and 4) a whole lapillus (polished on one side WL-1).

Materials and methods

First increment formation

Six male and six female spot were induced to spawn by injection of human chorionic gonadotropin (HCG) hormone at the NOAA Beaufort Laboratory. Eggs were incubated in a 100-L

Manuscript submitted 14 May 2004 to the Scientific Editor's Office.

Manuscript approved for publication 29 March 2005 by the Scientific Editor.

Fish. Bull. 103:544–552 (2005).

tank at constant temperature (20°C) and salinity (30‰), under 12 h light:12 h dark photoperiod. These conditions were maintained throughout the rearing period. Hatching occurred three days after spawning. Larvae were fed rotifers throughout the experiment and supplemented with enriched *Artemia* from day 20 through day 30. Larvae were collected 4 days (n=5), 12 days (n=7), and 27 days (n=5) after hatching, and live total length (L_T) measurements were made. Larvae were then preserved in 95% ethanol.

Sagittae and lapilli were dissected with fine-tipped forceps and embedded on microscope slides. The increments were clearly visible and otoliths did not require any additional preparation. All increment counts were conducted three times by one person on different occasions with a 100× oil objective and a Nikon E600 microscope with transmitted light. The light was polarized to obtain better visibility. The reader did not know the ages of the fish.

Known fish age and the number of observed increments were used to determine the time of first increment formation on both the sagittae and lapilli. The number of increments deposited between sampling dates divided by elapsed days indicated periodicity of increment formation.

Accuracy and precision

The experimental protocol and conditions were the same as in the previous examination of first increment formation, except that fish were reared for 53 days and artificial diet was added after day 30. Larvae (n=24, 8.8–16.1 mm L_T, mean=11.8 mm L_T) were collected 34 days after hatching, and juveniles (n=34, 19.4–28.1 mm L_T, mean=24.3 mm L_T) were collected 53 days after hatching.

Sagittae and lapilli were dissected from fish with fine-tipped forceps and embedded for sectioning on the transverse plane (right sagitta) or polishing on the sagittal plane (left whole sagitta and lapillus). Priority was given to transverse sections, and if the right sagitta was damaged during preparation, the left sagitta was used (n=8). Otoliths were sectioned with a slow-speed saw with dual diamond wafering blades. Sections were then ground on one side with 1000-grit sandpaper and polished with 0.3-μm alumina paste. After increments were counted on the proximal side of sections that were polished on one side (see below for details), sections were flipped over, ground, and polished to the core to provide a section that was polished on two sides. The left whole sagitta and lapillus were ground and polished in the sagittal plane with 0.3-μm alumina paste. One person made all the increment counts three times for each preparation technique on different occasions with a 100× oil objective on a Nikon E600 microscope with transmitted light. The reader knew the study design, but not the ages of the fish.

The mean number of increments counted from sagittae and lapilli prepared with different techniques were compared with known ages to determine the accuracy of the different aging methods. The statistical significance of differences in increment counts (accuracy) was evaluated with a one-way ANOVA. Increment formation rate was determined by comparing the number of increments counted to known age, and by comparing the difference in the number of increments between 34- and 54-day-old fish and the number of actual days between these increments (20 days).

Precision of increment counts from different otoliths and preparation techniques was determined with the coefficient of variation (CV), calculated by using the three increment counts made for each individual type of otolith and preparation technique (Chang, 1982). The differences in CV values among the four age estimation methods were analyzed by using a Kruskal-Wallis ANOVA. The statistical significance of observed differences were estimated with a *post hoc* Tukey HSD for unequal n test. All the statistical data analyses were performed with Statistica 6.0 software (StatSoft Inc., Tusla, OK).

Results

First-increment formation

First-increment formation on the sagitta occurred at hatching, but there may be problems in resolving increments near the core. Increment counts on sagittae were variable for 4-day-old larvae. Four increments were visible on the sagittae of one individual. The first increment was more pronounced than the others and was interpreted as a hatching check. This increment was approximately 8 μm from the core. On the sagittae of the remaining four 4-day-old larvae, only one increment was visible corresponding to the location of the perceived hatching check. Despite the apparent nondaily increment formation in 4-day-old larvae, an average of 12.3 (range 12–13) increments were visible on the sagittae of 12-day-old larvae, and an average of 26.5 (range 26–27) were visible on the sagittae of 27-day-old larvae. The first increment observed on the sagittae of 12- and 27-day-old larvae corresponded to the location of the first increment observed in the sagittae of 4-day-old larvae (Fig. 1).

First increment formation on the lapillus occurred 6–7 days after hatching. No increments were visible on the lapilli of 4-day-old larvae. In older larvae, an average of 6.4 (range 6–7) increments were observed on 12-day-old larvae and an average of 20.3 (range 20–21) increments were observed on the lapilli of 27-day-old larvae. Additionally, lapilli of 12 and 27-day old larvae exhibited two checks in the area between the otolith core and the first increment, but it was difficult to distinguish which check, if either, was formed at hatching (Fig. 1).

Accuracy and precision

Increments were clearly visible regardless of otolith preparation technique (Fig. 2). Increment width increased from

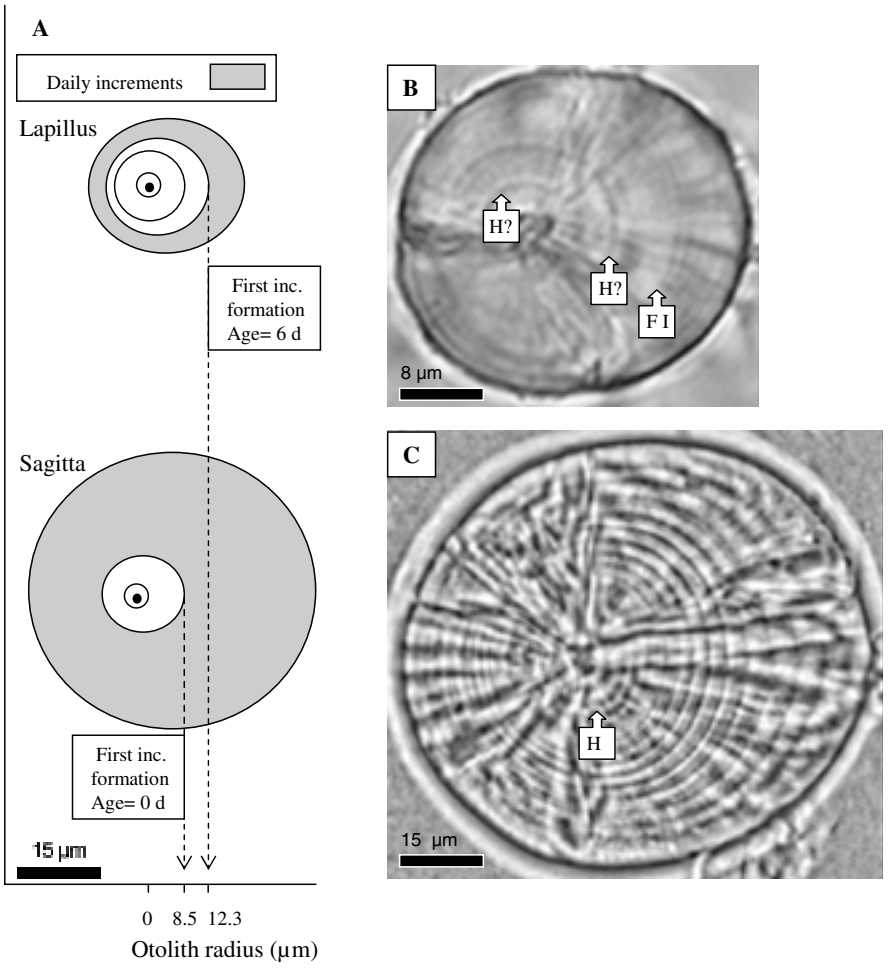

Figure 1

Diagram describing hatching check deposition as well as initiation of daily otolith increment formation in the sagittae and lapilli of spot (*Leiostomus xanthurus*) (**A**). Photographs of the otoliths of a 12-day-old larva: lapilli with six increments (**B**) and sagitta with 12 increments (**C**). Scale bar = 8 μm. H = hatching check; FI = first increment.

the core towards the otolith edge. In both sagittal preparations, increment counts could not easily be made along one radius owing to changes in the growth trajectories (Fig. 2A) and to discontinuities in increment formation (Fig. 2B). However, increment counts could be made along one radius in the lapillus (Fig. 2C)—an advantage that may facilitate measurements of otolith increment widths in future studies.

A hatching check was identified in the sagittae of 34-day-old larvae and 54-day-old juveniles at a location approximately 8.4 μm radius from the core (Table 1). In addition to the hatching check, another well-defined increment was observed in the core area of the sagittal otoliths (Fig. 3A), and this second check was likely related to a dietary switch to exogenous feeding. In most fish the second check was separated from the hatching check by an average of 5.2 increments (n=49, SD=0.59). However, in some fish (n=9), no increments were visible between the hatching check and the other well-defined increment. This observation indicates that there may be problems resolving increments near the core, similar to the results presented above regarding the timing of first increment formation. Owing to the apparent problems discerning increments near the core, the second check was used as a starting point for increment counts. Using the second check as a starting point influenced accuracy but provided a clear starting point for increment counts in all sagittal otolith preparations.

In the lapilli, increment deposition began from a pronounced check visible at ca. 12.3 μm radius from the otolith core (Fig. 3B). This check was found at the same distance from the core in lapilli of 12- and 27-day-old larvae in the experiment on first-increment formation (Table 1). Beginning increment counts from this check would underestimate age by 6–7 days owing to the timing of first-increment formation on the lapilli.

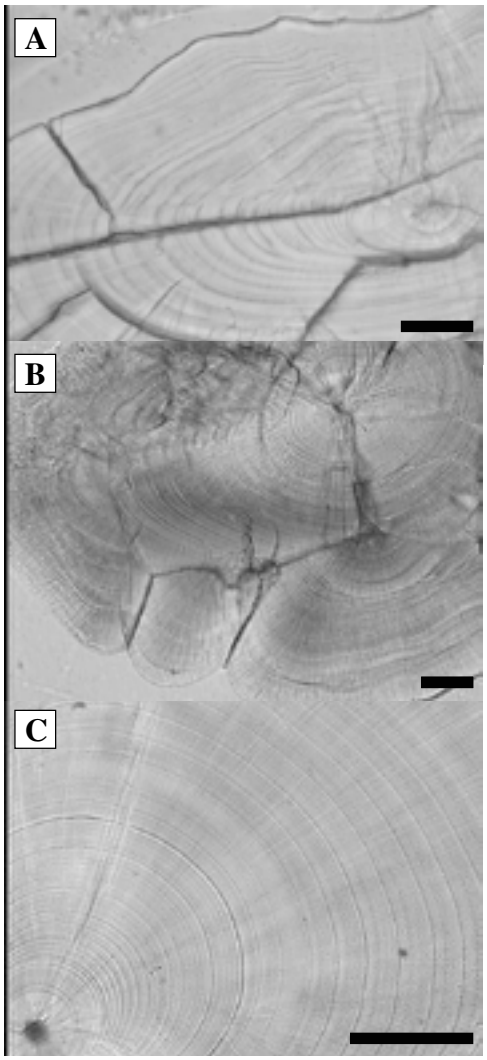

Figure 2

Otolith microstructure of an early-juvenile laboratory-reared spot (*Leiostomus xanthurus*): (**A**) transverse section of the sagitta (polished on two sides); (**B**) whole sagitta (polished on one side); and (**C**) whole lapillus (polished on one side). Scale bar = 30 μm.

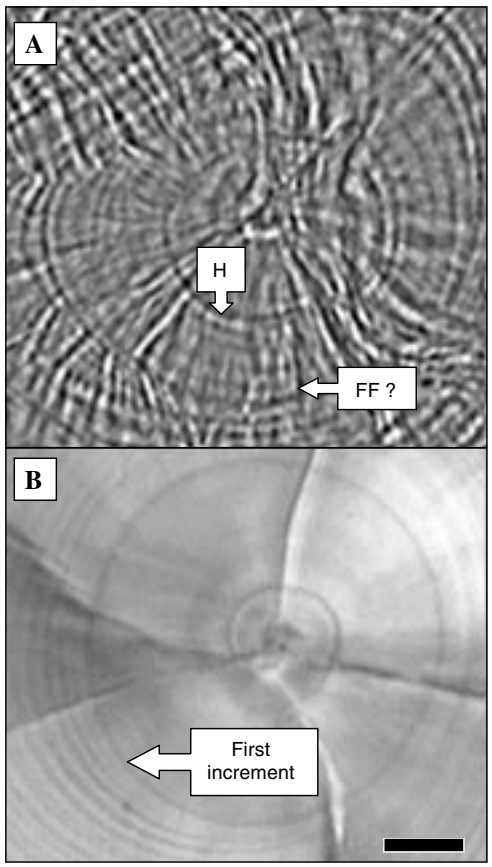

Figure 3

Central otolith area of early-juvenile laboratory-reared spot (*Leiostomus xanthurus*): (**A**) transverse section of sagitta (polished on two sides); five increments are visible between hatching check (H) and, presumably, first feeding check (FF); (**B**) whole lapillus (polished on one side) with daily increments deposited after the check was formed six days after hatching. Scale bar = 10 μm.

Increment formation occurred daily in both sagittae and lapilli after the early larval period. The difference in number of increments counted from sagittae and lapilli from fish sampled 34 and 53 days after hatching reflected the time elapsed between these two samplings (Table 2) and indicated daily increment formation between the larval and early juvenile stage. The same daily increment formation was also observed for larvae sampled 12 and 27 days after hatching during the experiment on first-increment formation (Table 2).

The accuracy of larval age estimates were similar for all the sagittae and lapilli preparation methods (ANOVA, $P>0.05$; Fig. 4A). For juveniles, however, there was a significant difference in the number of counted increments among sagitta preparation methods (ANOVA, $P<0.001$) (Fig. 4B). A lower number of increments were enumerated from transverse sections of sagittae (with one side polished) (*post hoc*: Tukey HSD for unequal n, $P<0.001$). Moreover, ~25% of otoliths within this group were not readable.

All the otolith preparation techniques, except the P1S transverse sections of sagitta from juveniles, underestimated the age from hatching by 9–10 days. A 6–7 day difference was expected between known age and lapilli increment counts, owing to the time of first-increment formation. Thus, actual fish age was underestimated by approximately 2–4 days with lapilli increment counts. A 5-day difference was expected between known age and

Table 1

The distance from otolith core to first increment in the sagitta (first increment formed on the first day after hatching) and in the lapilli (first increment formed six days after hatching) of laboratory-reared spot (*Leiostomus xanthurus*).

		Distance to the first increment (μm)		
Otolith	*n*	Mean	SD	Range
Sagittae—experiment on first-increment formation	17	8.3	0.76	6.7–9.9
Sagittae[1]—experiment on accuracy and precision of aging technique	36	7.8	0.91	6.7–8.8
Lapilli—experiment on first-increment formation	17	12.3	0.54	11.5–13.2
Lapilli—experiment on accuracy and precision aof aging technique	25	12.2	0.61	11.0–14.2

[1] Data for both whole sagittae (polished on one side) along sagittal view, and transverse sections of sagittae polished on two sides.

Table 2

Number of increments deposited on the otoliths of laboratory-reared spot (*Leiostomus xanthurus*) between sampling days in comparison with number of days between sampling days.

Otolith	Sampling days (days after hatching)	Days between sampling	Number of increments between sampling[2]
Sagittae—experiment on first-increment formation	12 and 27	15	14.3
Sagittae[1]—experiment on accuracy and precision of age determination	34 and 53	19	18.3
Lapilli—experiment on first-increment formation	12 and 27	15	14.1
Lapilli—experiment on accuracy and precision of age determination	34 and 53	19	18.6

[1] Data for both whole sagittae (polished on one side) and for transverse sections of sagittae (polished on two sides).

[2] No variance is given because the value represents difference between two average increment numbers obtained for two different groups of fish.

whole-sagittae increments counts, owing to the initiation of increments from a second check, which formed approximately 5 days after hatching. With whole-sagittae increment counts, actual fish age was underestimated by approximately 5 days.

The coefficients of variation (CV), which indicates the precision of age estimates, varied from 1.4% to 8.3% (Fig. 5). CVs were statistically different among age estimation methods for both larvae and juveniles (Kruskal-Wallis ANOVA, $P<0.001$). Lapilli from both larvae and juveniles had lowest CVs, indicating high precision. Whole sagittae and P2S transverse sections for juveniles were comparable, but lower precision for larvae was observed. However, if transverse sections are used for aging, the preparation of both sides is important in the case of larvae (with regard to precision; see Fig. 5) and mandatory in the case of juveniles (with regard to accuracy; see Fig. 4B). In addition, the confidence of the otolith reader in increment recognition (Fig. 5) indicated that the most clear and easy to count increments were found in the lapilli.

Discussion

First-increment formation

In prior studies, the age of larval and juvenile spot was estimated by adding five days to the number of increments counted from sagittae (e.g., Warlen and Chester, 1985; Flores-Coto and Warlen, 1993; Ross, 2003). Our research indicated that increment formation in sagittae occurred at hatching. The only study validating first-increment formation in spot used linear regression analysis for laboratory-reared fish (Peters et al.[1]). The intercept of their regression line (age in relation to number of increments) indicated that the first increment

[1] Peters, D. S, Jr, J. C. DeVane, M. T. Boyd, L. C. Clements, and A. B. Powell. 1978. Preliminary observations on feeding, growth and energy budget of larval spot (*Leiostomus xanthurus*). *In* Ann. Rep. Southeast Fish. Cent., Beaufort Lab. to U.S. Dep. Energy, p. 377–397. Beaufort Laboratory, National Marine Fisheries Service, Beaufort, NC.

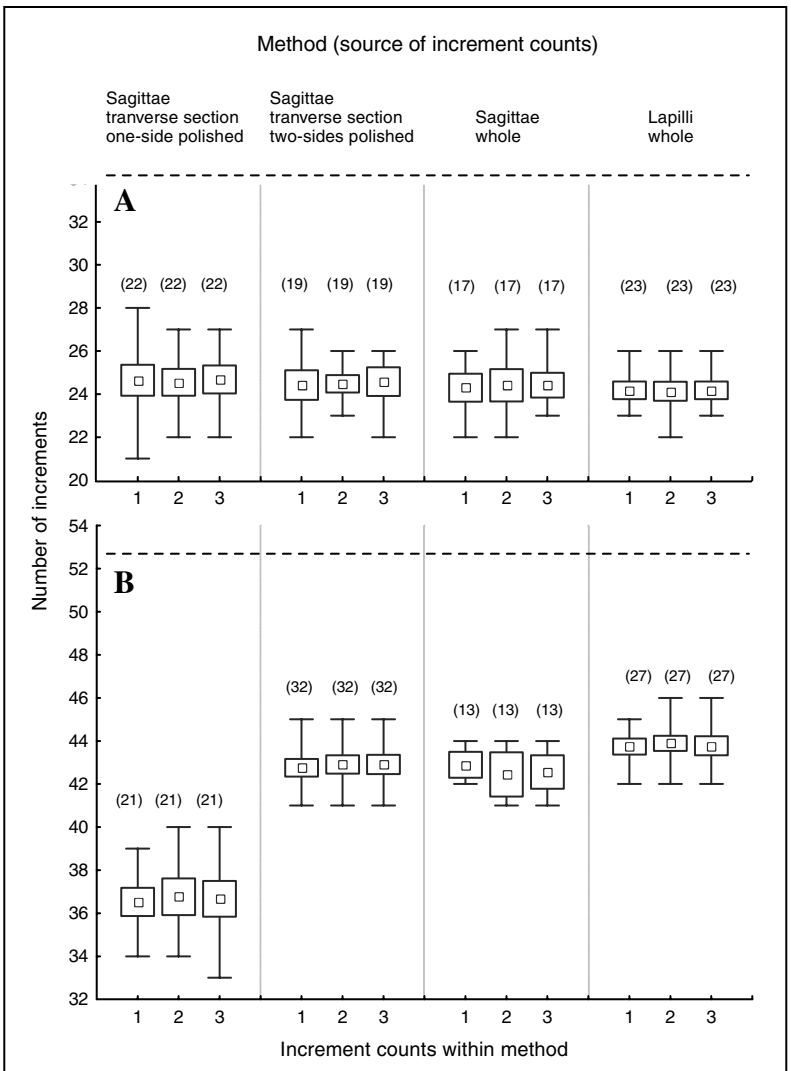

Figure 4

Age of laboratory-reared spot (*Leiostomus xanthurus*) estimated from daily otolith growth increments counted at three different occasions for each preparation method: (**A**) larvae (34 d, 11.8 mm L_T); and (**B**) juveniles (53 d, 24.3 mm L_T). Mean and 95% confidence interval minimum, and maximum values are presented. Values in parentheses indicate sample number. Dashed line indicates the real age.

formed five days after hatching, which corresponds to a time of exogenous feeding initiation in spot (Powell and Gordy, 1980; Powell and Chester, 1985). The other validation experiments on spot (Hettler, 1984; Siegfried and Weinstein, 1989) provided no information on first increment deposition time. In lapilli, increment deposition occurred six days after hatching, but no other studies are available for spot to compare and evaluate these results.

The inconsistency in the time of first increment formation on the sagittae between the present study and Peters et al.'s study[1] may be the result of underestimation by the latter because they did not section or polish the otoliths. Spot otoliths are relatively large and thick and both sagittae and lapilli are difficult to read without otolith preparation for fish older than 25–30 days (~7–9 mm TL). Peters et al.[1] found no increments in sagittae of four- to five-day-old fish. Although in the present study increments were not clear in sagittae of four-day-old spot, fish collected from the same tanks, 8 and 23 days later, had visible increments since hatching. Even if it is difficult to explain why the increments in sagittae of four-day-old-fish were not visible, results presented in the present study support the conclusion that first increment formation occurred at hatching.

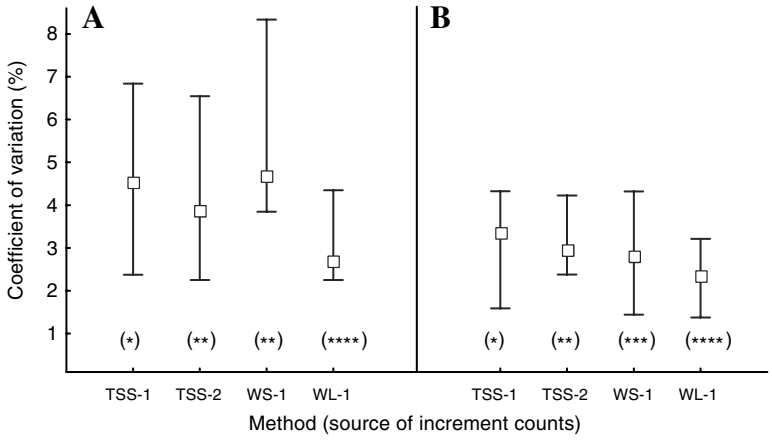

Figure 5

Precision evaluation for different aging methods employed for larval (**A**) and early-juvenile (**B**) laboratory-reared spot: transverse section of sagitta (polished on one side) (TSS-1), transverse section of the sagitta (polished on two sides) (TSS-2), whole sagitta (polished on one side) (WS-1), and a whole lapillus (polished on one side) (WL-1). The coefficients of variation (CV) values were calculated for three independent increment counts per otolith. Additionally, the confidence of the otolith reader in increment recognition has been indicated by numbers of stars, i.e., poor (*), relatively good (**), good (***), and very good (****).

Accuracy and precision of age estimates among different types of otoliths and preparation techniques

Lack of distinct patterns in daily growth increments in otoliths of laboratory-reared fish (e.g., David et al., 1994) could make it difficult to conduct laboratory-based ecological experiments with larval fish. Hettler (1984) attempted to validate increment formation rate in the sagittal otoliths of laboratory-reared spot (13–16 mm SL). Within eight days after tetracycline marking, otolith radii increased approximately 18%, but no increments were observed. Siegfried and Weinstein (1989) confirmed daily increment formation in the sagittae of field-reared spot larvae, but those reared in the laboratory produced 17 increments instead of the expected 30. Our results, on the other hand, provided direct validation of daily increment formation in the sagittae and lapilli of laboratory-reared spot (Table 2).

Even though increment formation was found to occur daily, there were inaccuracies in the estimate of age from otolith increment counts. Twenty-four increments were counted on the sagittae of 34-day-old larvae; if five increments were added for time between first-increment formation and formation of the second check (the starting point of counts used in the present study), age was still underestimated by 4–5 days. Similarly, 24 increments were counted on the sagittae of 34-day-old larvae; if 6–7 days were added to account for the timing of increment formation in the lapillus, age was underestimated by 3–4 days. Similar inaccuracies in age estimates were derived for 53-day-old juveniles. Peters et al.[1] also found age inaccuracies of five days from sagittal increments and concluded that first-increment formation occurred five days after hatching. Given our results and those of Hettler (1984) and Siegfried and Weinstein (1989), we conclude that the likely explanation for age inaccuracies is that the increments near the core of the otolith become harder to read as more otolith material is laid down and this process results in the appearance of fewer increments. These inaccuracies would contribute to a 10–15% underestimation of age from sagittae and a 3–11% underestimation of age from lapilli. To account for these inaccuracies, five increments should be added to increment counts to estimate age.

Lapilli, compared with sagittae, exhibited very clear patterns with increments (Fig. 2) and provided more precise results for the ages of larval and juvenile spot. Although there is no study presenting age data obtained from lapilli for larval or juvenile spot, lapilli have been used successfully for aging many other fish species. Ichimaru and Katsunori (1995) preferred the lapillus as a source of age data for two species of flyingfishes larvae (*Cypselurus heterurus doederleini* and *Cypselurus hiraii*) because increments were as clear as those in the sagittae, yet the lapilli did not require any preparation. Bestgen and Bundy (1998) reported increments deposited on sagittae of Colorado squawfish (*Ptychocheilus lucius*) were difficult to distinguish after fish were 30 days old and thus lapilli were used to age older fish. Lapilli were the preferred otoliths for age determination of young Lost River sucker (*Deltistes luxatus*) and shortnose sucker (*Chasmistes brevirostris*) because of their readability and conservative growth pattern (Hoff et al., 1997). Escot and Grando-Lorencio (1998) concluded

that increments in lapilli of *Barbus sclateri* (Pisces: Cyprinidae) were more clearly defined than in sagittae and asterisci. Similarly, our results demonstrate the utility of lapilli for larval and juvenile fish age estimates.

In addition to the choice of the most suitable type of otolith, the choice of the most appropriate preparation method is an important aspect of larval and juvenile fish age determination (Secor et al., 1992). Analysis of P1S whole sagittae provided in the current study similar precision and confidence in age determination as transverse sections. Although analysis of sagittal transverse sections have been applied to spot (Siegfried and Weinstein, 1989), the most frequently used method has been the analysis of whole sagittae in sagittal view (Hettler, 1984; Warlen and Chester, 1985; Powell et al., 1990; Flores-Coto and Warlen, 1993; Ross, 2003). Recently, Ross (2003) was able to age 40–160 day-old spot juveniles, analyzing whole sagittae along the sagittal view; however, polishing on both sides was frequently necessary. For whole lapilli, however, only one preparation method (i.e., polishing along the sagittal plane) was used in the present study and the results were more satisfactory then those obtained for sagittae and hence no other preparation method (i.e., sectioning) seemed to be required.

In conclusion, first-increment formation occurs at hatching in the sagittae and at 6–7 days after hatching in the lapilli. Increment formation rate occurs daily in both the sagittae and the lapilli. With sagittal and lapillar increment counts, age was underestimated and the cause appeared to be difficulty in discerning increments near the core. Whole lapilli (prepared by polishing one side along the sagittal section) provided age accuracy similar to that of the three sagittal preparations, but higher precision. Future studies would benefit from using the lapillus for ecological studies of the early life history of spot.

Acknowledgments

The authors thank Elisabeth Laban for consultation during otolith preparation and analysis, as well as Dean Ahrenholz and Jennifer Potts for reviewing the earlier version of the manuscript. This research was performed while the first author held a National Research Council Research Associateship Award at the NOAA Beaufort Laboratory.

Literature cited

Barkmann, R. C., and A. Beck.
1976. Incubating eggs of the Atlantic silverside on nylon screen. Prog. Fish-Cult. 38:148–150.

Bath Martin, G. E., and S. R. Thorrold.
2005. Temperature and salinity effects on magnesium, mananese, and barium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). Mar. Ecol. Prog. Ser. 293:233–240.

Bath Martin, G. E., S. R. Thorrold, and C. M. Jones.
2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). Can. J. Fish. Aquat. Sci. 63:34–42.

Bath Martin, G. E., S. R. Thorrold, C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. H. Lam.
2000. Strontium and barium uptake in aragonitic otoliths of marine fish. Geochim. Cosmochim. Acta 64:1705–1714.

Bestgen, K. R., and J. M. Bundy.
1998. Environmental factors affect daily increment deposition and otolith growth in young Colorado squawfish. Trans. Am. Fish. Soc. 127:105–117.

Campana, S. E.
2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation method. J. Fish Biol. 59:197–242.

Campana, S. E., and E. Moksness.
1991. Accuracy and precision of age and hatch date estimation from otolith microstructure examination. ICES J. Mar. Sci. 48:303–316.

Campana, S. E., and J. D. Neilson.
1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42:1014–1032.

Chang, W. Y. B.
1982. A statistical method for evaluating the reproducibility of age determination. Can. J. Fish. Aquat. Sci. 39:1208–1210.

David, A. W., J. J. Isley, and C. B. Grimes.
1994. Differences between the sagitta, lapillus, and asteriscus in estimating age and growth in juvenile red drum, *Sciaenops ocellatus*. Fish. Bull. 92:509–515.

Escot, C., and C. Granado-Lorencio.
1998. Morphology of the otoliths of *Barbus sclateri* (Pisces: Cyprinidae). J. Zool. 246:89–94.

Flores-Coto, C., and S. M. Warlen.
1993. Spawning time, growth, and recruitment of larval spot *Leiostomus xanthurus* into a North Carolina estuary. Fish. Bull. 91:8–22.

Geffen, A. J.
1987. Methods of validating daily increment deposition in otoliths of larval fish. *In* Age and growth of fish (R. C. Summerfelt and G. E. Hall, eds.), p. 223–240. Iowa State Univ. Press. Ames, IA.

Govoni, J. J., A. J. Chester, D. E. Hoss, and P. B. Ortner.
1985. An observation of episodic feeding and growth of larval *Leiostomus xanthurus* in the northern Gulf of Mexico. J. Plank. Res. 7:137–146.

Govoni, J. J., and D. E. Hoss.
2001. Comparison of the development and function of the swimbladder of *Brevoortia tyrannus* (Clupeidae) and *Leiostomus xanthurus* (Sciaenidae). Copeia 2001(2):430–442.

Hettler, W. F.
1984. Marking otoliths by immersion of marine fish larvae in tetracycline. Trans. Am. Fish. Soc. 113:370–373.

Hoff, G. R., D. J. Logan, and D. F. Markle.
1997. Otolith morphology and increment validation in young Lost River and shortnose suckers. Trans. Am. Fish. Soc. 126:488–494.

Ichimaru, T., and T. Katsunori.
1995. Otolith increment formation of flyingfishes larvae, *Cypselurus heterurus doederleini* and *Cypselurus hiraii*

under rearing conditions. Bull. Nagasaki Prefect. Inst. Fish. 21:1–6.

Jones, C. M.
1986. Determining age of larval fish with the otolith increment technique. Fish. Bull. 83:289–298.

Mercer, L. P.
1987. Fishery management plan for spot (*Leiostomus xanthurus*). ASMFC Fishery Management Report 11:1–81. Atlantic States Marine Fisheries Commission, Washington, D.C.

Morioka, S., and L. Machinandiarena.
2001. Comparison of daily increment formation pattern between sagittae and lapilli of ling (*Genypterus blacodes*) larvae and juveniles collected off Argentina. N.Z.J. Mar. Freshw. Res. 35:111–119.

Neilson, J. D., and G. H. Geen.
1985. Effects of feeding regimes and dial temperature cycles on otolith increment formation in juvenile salmon (*Occorhynchus tschawytscha*). Fish. Bull. 83:91–101.

Powell, A. B., and A. J. Chester.
1985. Morphometric indicis of nutritional conditions and sensitivity to starvation of spot larvae. Trans. Am. Fish. Soc. 114:338–347.

Powell, A. B., A. J. Chester, J. J. Govoni, and S. M. Warlen.
1990. Nutritional condition of spot larvae associated with Mississippi River Plume. Trans. Am. Fish. Soc. 119:957–965.

Powell, A. B., and H. R. Gordy.
1980. Egg and larval development of the spot, *Leiostomus xanthurus* (*Sciaenidae*). Fish. Bull. 78:701–714.

Radtke, R. L., and J. M. Dean.
1982. Increment formation in the otoliths of embryos, larvae, and juveniles of the mummichog, *Fundulus heteroclitus*. Fish. Bull. 80:201–215.

Ross, S. W.
2003. The relative value of different estuarine nursery areas in North Carolina for transient juvenile marine fishes. Fish. Bull. 101:384–404.

Secor, D .H., J. M. Dean, and E. H. Laban.
1991. Otolith removal and preparation for microstructural examination: a user's manual. Technical Publ. 1991-01, 85 p. The Electronic Power Research Institute and the Bell W. Baruch Institute for Marine and Coastal Sciences, Columbia, SC.

Siegfried, R. C. II, and M. P. Weinstein.
1989. Validation of daily increments deposition in the otolith of spot (*Leiostomus xanthurus*). Estuaries 12:180–185.

Thorrold, S. R., and J. A. Hare.
2002. Otolith applications in reef fish ecology. *In* Coral reef fishes: dynamics and diversity in a complex ecosystem (P. F. Sale, ed.), p. 243–264. Academic Press, San Deigo, CA.

Walter, J. F., and H. M. Austin.
2003. Diet composition of large striped bass (*Morone saxatilis*) in Chesapeake Bay. Fish. Bull. 101:414–423.

Warlen, S. M., and A. J. Chester.
1985. Age, growth, and distribution of larval spot, *Leiostomus xanthurus*, off North Carolina. Fish. Bull. 83:587–599.