Periodicity of increment formation in otoliths of overwintering postlarval and prejuvenile Atlantic menhaden, *Brevoortia tyrannus*

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Hypotheses of fishery recruitment processes often have as their foundation studies of incremental otolith growth (e.g. Methot, 1983; Rice et al., 1987). Conclusions from these studies, however, are only as robust as their baseline otolith validation work. This is especially true for investigations on Atlantic menhaden (*Brevoortia tyrannus*), an estuarine-dependent, marine-migratory clupeid ranging from southeastern Florida to Nova Scotia (Reintjes, 1969; Ahrenholz, 1991). The spawning season for Atlantic menhaden is protracted, occurring wherever fish of spawning age are found (Judy and Lewis, 1983). Spawning is probably greatest from October to March (Higham and Nicholson, 1964). The spawning activity that occurs in south coastal U.S. waters normally occurs within this time period, usually peaking during mid- to late winter (Wilkens and Lewis, 1971; Nicholson, 1972). Owing to the extended spawn and seasonal movement of spawning adults, progeny from different temporal spawning periods experience different environmental conditions during similar early life history stages.

Daily age validation studies have been conducted on late larvae and juvenile Atlantic menhaden (Ahrenholz et al., 1995), but none of the specimens were reared in a water temperature regime that typified conditions experienced by fall-spawned larvae. Fall-spawned larvae enter inshore nurseries when estuarine waters are cooling, yet still warm enough for moderate somatic growth and development. These young fish then endure the coldest winter temperatures in a partially transformed condition, i.e. in a postlarval-prejuvenile stage. In contrast, larvae of winter and early spring spawnings ingress into relatively cold, but progressively warming waters, from late winter to midspring; although they probably do not enter during extreme winter cold spells (Reintjes and Pacheco, 1966). Differences in growth patterns in the otolith microstructure of larvae and juveniles from these different spawning periods are detectable (Fitzhugh et al., 1997).

Validation of the otolith daily aging technique should take into account the environmental conditions experienced by field-sampled specimens (Jones, 1986), especially when some field conditions affect the daily periodicity of otolith growth increments. The sagittal growth increments for the fall-spawned Atlantic menhaden are disproportionately narrower (±1 μm) during the coldest overwintering months. Moreover, increments <1 μm potentially indicate less than daily increment formation (Campana et al., 1987). Less than daily increment deposition associated with cold water and slow growth rates has been observed for juvenile summer flounder (*Paralichthys dentatus*), another estuarine-dependent, marine-migratory species (Szedlmayer and Able, 1992).

Previous otolith microstructure validation studies for larval Atlantic menhaden have used larvae collected in the field during April or laboratory-reared specimens spawned in February (Ahrenholz et al., 1995). Test conditions were set at higher salinities than are normally associated with larval to juvenile transformation. Hence, we designed a study to specifically address the periodicity of otolith increment formation in over-wintering, fall-spawned Atlantic menhaden postlarvae and prejuveniles under the thermal and salinity conditions they experience in North Carolinian estuaries.

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Materials and methods

Atlantic menhaden larvae were collected while ingressing into the Newport River estuary during the week of 7 December 1994. In the laboratory, these larvae were immersed on 8 December, in 100 mg/L solution of alizarin-complexone (ALC) solution for 16 h (overnight) at 18°C. Nearly equal numbers of marked larvae were reared in two concrete ponds (6600 L). Pond one, the high-salinity pond, received flow-through seawater at ambient water temperatures. Pond two, the low-salinity pond, received flow-through seawater and freshwater from a well. Pond two was generally a few degrees warmer and about one-half the salinity of pond one. Menhaden larvae were fed powdered salmon starter, supplemented with brine shrimp larvae, for the first few weeks, followed by salmon starter only.

Two to six specimens were sacrificed periodically, and measured for fork length (FL) to the nearest mm. Sagittal otoliths were removed, mounted in droplets of epoxy resin, and sectioned obliquely with a slow-speed saw with dual diamond wafering blades (Ahrenholz et al., 1995). Polished otolith sections (10–15 µm thick) were viewed through a compound microscope (1000×) and on an image analysis video monitor (3800×). When the otolith sample was examined under blue light epifluoresence, the ALC-marked increment was seen as red-orange. The number of increments from the ALC mark to the otolith margin was estimated as the arithmetic mean of a series of independent counts tallied blindly with a hand-held counter. A section was counted twice when the two counts differed by 0 to 2 increments. A section was counted three to five times when the difference between the initial two counts was greater than two increments. Otoliths from one specimen from the high-salinity pond were deemed unreadable.

An image analysis system was used to measure increment widths in groups of 10, starting from the innermost increment, for a select number of otoliths from each test group. However, the increments measured just prior to the alizarin mark may have numbered from 5 to 10. This procedure was undertaken to separate pre- and postmark increments from width estimates. Measurements were taken from oblique-transverse sections (Ahrenholz et al., 1995) as opposed to similar measurements taken on transverse sections, as done by Fitzhugh et al. (1997). Owing to nearly concentric otolith growth for the life history stage sampled, the measurements taken in our study should be similar to, or slightly greater than, transverse-oriented measurements.

Estimates of the dates when eggs were spawned for the test groups were obtained by sacrificing eight larvae on the day of ALC-marking. Their otoliths were removed, mounted on microscope slides, and read whole. Three independent counts were made from the first increment following the first feeding mark to the margin. Five days were added to the mean counts to account for time from spawning to first increment formation (Warlen, 1992).

Statistical analyses of the results generally followed those of Ahrenholz et al. (1995). The null hypothesis tested was that the growth increments in the otoliths were formed daily during the rearing period. The hypothesis was not rejected if the slope of a regression of increment count on number of days since marking was not significantly different from one and the intercept was not significantly different from zero. The statistical power to test for differences in the slope was also calculated (Rice, 1987). Student's-*t* test was used to test for statistical significance of the regression parameters. Analysis of covariance (ANCOVA) and regression computations were performed with SAS statistical programs (SAS Institute, 1985). Ninety-five percent confidence intervals (CI) for individual age estimations (see discussion in Rice [1987]) were calculated for an inverse prediction condition (Sokal and Rohlf, 1981; note also discussions of inverse regression in Draper and Smith [1980]).

Results

Fish were sampled from pond two (the low-salinity regime) on postmarking days 35, 56, 74 (and 75), 98, 119, and 140 (total n=22). Fish from pond one (the high-salinity regime) were sampled similarly through day 75 (total n=13). Prior to day 75 (for about two weeks), many of the menhaden in pond one died. The remaining fish from the high-salinity regime were sampled on day 75.

Spawned-date estimates determined from larvae sacrificed before being reared ranged from 17 October to 20 October 1994, thus, verifying that the experimental tests were performed on larvae derived from fall-spawning activity.

Temperature and salinity regimes during the rearing period (Fig. 1) were adequate to attain the study's objectives. The cooling and warming pattern, as well as the actual water temperatures were well within the range observed for winters in North Carolina estuaries over a 15-year period (Hettler and Chester, 1982). Salinity levels in both rearing regimes were within ranges observed for larval or juvenile menhaden in the field (Wilkenson and Lewis, 1971; Turner and Johnson, 1973), although the larval forms are more highly associated with lower salinities. Water temperatures were relatively warm in the early por-

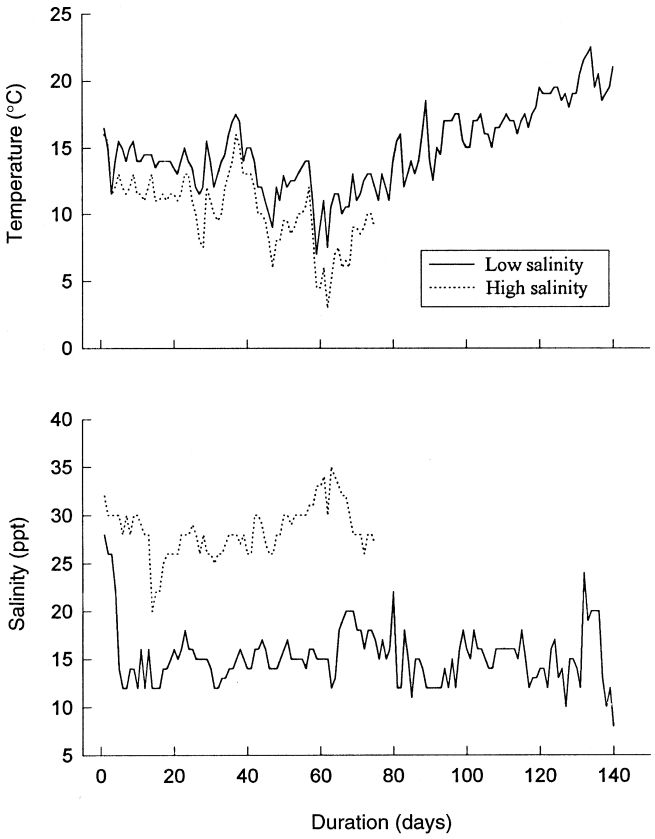

Figure 1

Environmental conditions experienced by two groups of ALC-marked larval-juvenile Atlantic menhaden (*Brevoortia tyrannus*) during the otolith validation study. The upper panel depicts temperature histories and the lower panel, the salinity histories. The high-salinity (low-temperature) rearing experiment ended after 75 days owing to high mortality rates.

tion of the study; larval menhaden advanced well into their transformation into juveniles. Through day 60 of rearing, water temperatures steadily declined to low winter minima. During the lower temperature period, the growth rates of fishes were reduced (Fig. 2). The mean width of the otolith growth increments for fish from the high-salinity group gradually dropped below 1 mm following the severe drop in temperature. During the same period, the increments for the low-salinity group reflected the higher growth rates for this group and were wider, with all mean widths above 1 µm (Fig. 3).

Similarity between regression parameters of increment counts versus days since marking between the high- and low-salinity regimes was tested over the shared range of duration (75 days). Tests for homogeneity of slopes showed no differences (P=0.679). A subsequent ANCOVA analysis revealed no differences between intercepts (P=0.712). Thus, the increment counts for the two rearing regimes were pooled and validation hypotheses were tested with a regression analysis. Results of this analysis revealed that the intercept was not significantly different from zero, and the slope was not significantly different from one (Fig. 4, Table 1). The confidence interval (95%) for estimates of daily age obtained from otoliths of fish which encountered conditions similar to those tested here, would be ±9 to 10 days over a range of 140 days (approximately 50 to 190 days of age).

Discussion

Growth increments on sagittal otoliths of fall-spawned Atlantic menhaden formed daily, even when experimental water temperatures declined to 3°C

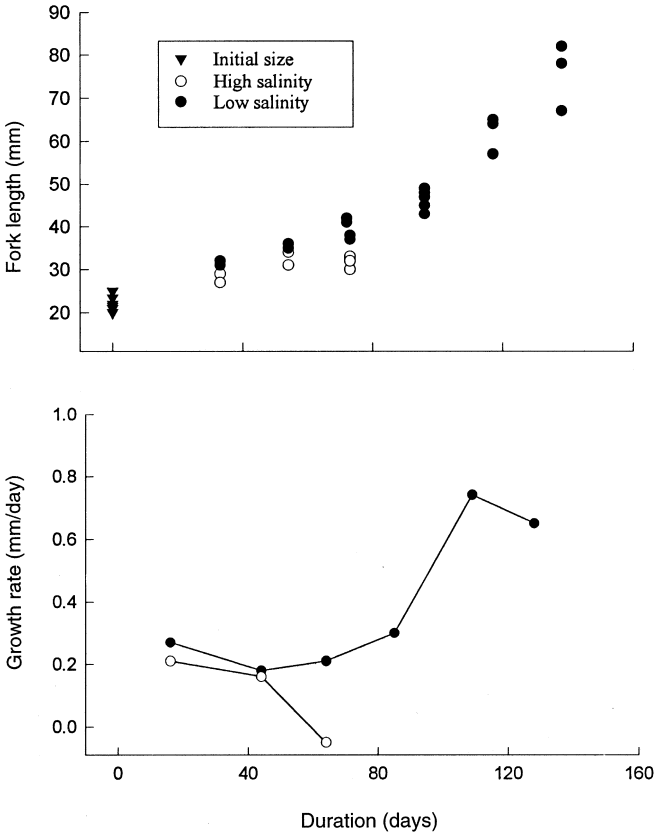

Figure 2

The body size (fork length) of ALC-marked juvenile Atlantic menhaden (*Brevoortia tyrannus*) sampled during the validation rearing study (upper panel) and rate of growth estimated from mean size of sampled fish (lower panel). The rates of growth are plotted at midpoints between sampling periods.

Table 1

Results of least squares linear regression analysis for marked Atlantic menhaden, *Brevoortia tyrannus*, otolith increment counts (n=34, r^2=0.984, and SE=standard error).

Parameter	Estimate	SE	P
Slope	1.0048	0.0224	0.168
Power[1]	99.1%	—	—
Intercept	0.3771	1.8257	0.162

[1] Statistical power to detect a deviation of 0.1 in the estimate of the slope at the P=0.05 level.

(Fig. 1). Even though growth rate declined to low levels during initial study weeks, especially for the high-salinity group (Fig. 2), an otolith increment continued to be formed daily even when increment widths fell below 1 µm for a number of days (Fig. 3). If a difference in increment formation rate had occurred between groups, we would not have been able to determine the cause because both temperature and salinity varied between groups. It seems likely that the faster growing fish responded to the higher temperatures. Regardless, both test groups experienced conditions that could be expected in the field during winter. The October-spawned larval menhaden used in our study displayed a growth increment mean width that ranged from values that were greater than those previously measured at sea to those that were less than previously measured in estuaries in a relatively short period of days (compare with Fitzhugh, et al. 1997).

The rearing conditions for specimens maintained during our study and earlier studies (Ahrenholz et al., 1995) should provide otolith increment validations for most, if not all, Atlantic menhaden that recruit

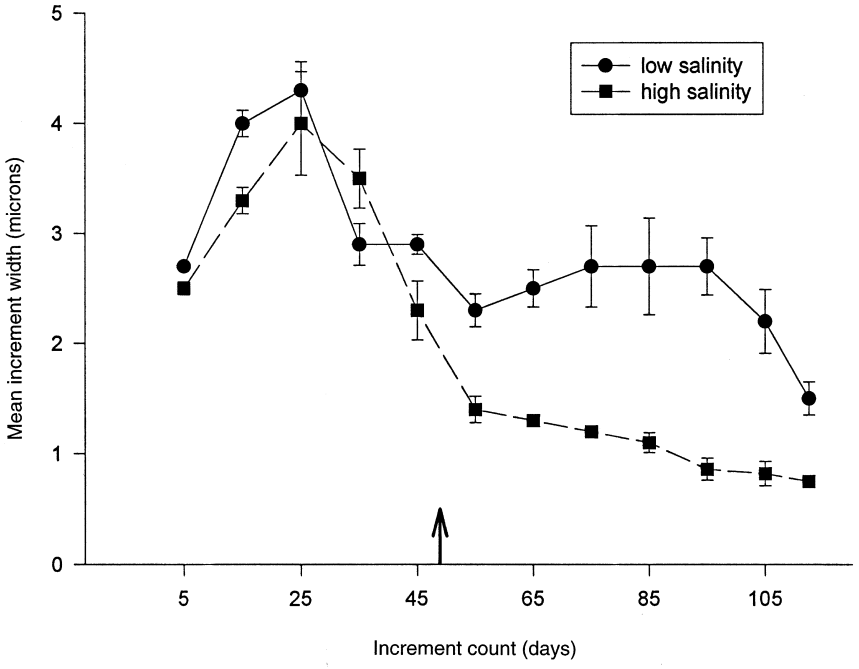

Figure 3

Estimates of mean width for otolith growth increments from larval-juvenile Atlantic menhaden (*Brevoortia tyrannus*) from each test-rearing group. The error bars represent ±1 SE, with n=3 for each data point (the error bars may be hidden by the symbol for some entries). The arrow denotes when the alizarin marking occurred. At-sea values are thus to the left of the arrow. (Note: after ALC marking, the low-salinity group was reared at a higher temperature than the high-salinity group.)

into estuarine systems from North Carolina to northern Florida. Additional studies may be necessary for more northern systems, such as Chesapeake and Delaware bays, especially for fall-spawned postlarval menhaden that may overwinter in even colder waters and for more extended periods of time.

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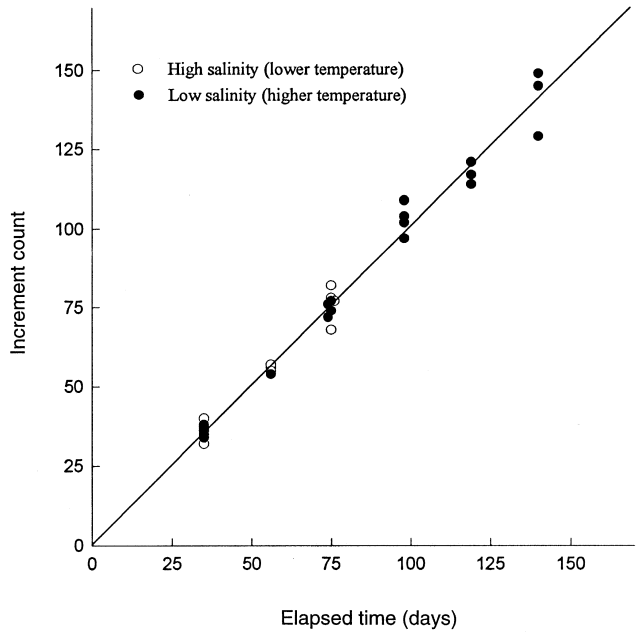

Figure 4

Scatterplot of growth increment count on elapsed time since ALC-marking for juvenile Atlantic menhaden (*Brevoortia tyrannus*). The fitted least squares regression line is shown.

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