VALIDATION OF THE OTOLITH INCREMENT AGING TECHNIQUE FOR STRIPED BASS, *MORONE SAXATILIS*, LARVAE REARED UNDER SUBOPTIMAL FEEDING CONDITIONS

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ABSTRACT

Striped bass, *Morone saxatilis*, larvae were reared in the laboratory for 97 days to validate the otolith increment aging technique for this species. Otolith-increment deposition rates were determined under optimal laboratory conditions for growth and under three conditions of restricted feeding and using both light and scanning electron microscopy (SEM). Under optimal laboratory conditions, increments were deposited daily from the fourth day after hatching through the first 2 months of life and were discernible with the light microscope. For larvae reared under restricted feeding regimes and readings done with the light microscope, counts did not reflect true age. Counts obtained from these same otoliths using SEM, however, more closely reflected true daily age. Results indicate that the use of light microscopy alone can result in inaccurate estimation of age for larvae that have experienced starvation episodes.

When otolith increments in larval fish are deposited daily, with a known time of onset, precise age of each individual can be determined and the growth curves for the individuals may be generated. The ability to follow changes in growth of individuals and populations on as fine a scale as, say, a week may provide a means to improved understanding of the effects which environmental factors have on survival.

To apply this aging technique to larval striped bass, *Morone saxatilis*, daily deposition of increments and the age at first increment deposition had to be confirmed in the laboratory with known-age larvae. Although daily depositional rates of otolith increments in known-age larval striped bass have not been previously reported, daily deposition has been noted for larvae and juveniles of 17 other species of fish reared in the laboratory (see Jones 1985 for review). Nonvalidated data exist to support the concept of daily increment deposition for field-captured striped bass (Brothers et al. 1976). However, tests of depositional rate under suboptimal laboratory conditions, using light microscopy, have shown that depositional rates can be affected by the specific growth rate (Geffen 1982), by photoperiod (Radtke 1978), by food supply (Geffen 1982; Neilson and Geen 1982), and by temperature (Brothers 1978; Geffen 1983). Campana and Neilson (1985) stated that "few workers have critically assessed the

assumptions upon which the age and growth inferences are based or considered the potential for environmental modification of microstructural features."

Of particular importance is the potential for counting fewer otolith increments when otolith growth rate is slowed to the extent that increments being deposited are too narrow to resolve with a light microscope. Inadequate resolution with the light microscope could lead to systematically low increment counts and thus, result in overestimation of the growth and mortality rates, and underestimation of variance in growth, all of which have important biological implications. Hence, to demonstrate that striped bass larvae from the field could be aged accurately by the otolith increment technique, we found it necessary to determine the regularity and readability of otolith-increment deposition under simulated laboratory suboptimal field conditions.

Lack of scanning electron microscopy (SEM) validation hinders the resolution of an important issue: Is daily formation of increments a robust biological rhythm common to most teleosts which requires serious and prolonged starvation to disrupt, or is it a more volatile physiological connection in which daily formation occurs only under optimal food concentrations as certain laboratory studies indicate?

Factors which affect growth and survival of striped bass larvae have been studied extensively (see Westin and Rogers 1978 for review). Rogers (1978) raised larval striped bass under various temperature and feeding regimes to determine growth under laboratory conditions. Larvae grew well at

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temperatures between 16° and 22°C and with a minimum of 1,000-2,000 *Artemia* nauplii/L. The optimum salinity range was between 3.5 and 14.0‰ (Bayless 1972). Davies (1973) studied larval survival under combinations of temperature, pH, and dissolved solids. Optimum temperature was 17.0°C; optimum pH, 7.5. Eldridge et al. (1981) studied the growth of larvae under various feeding regimes and found growth rates which approximated field growth rates at concentrations of 5,000 *Artemia*/L. They found that the "point of no return" was ill defined and starved larvae could live for as long as 31 days. Dey (1981) has reported on growth and survival of wild larvae, using length and developmental stage to estimate growth. He found growth was temperature dependent and temperatures between 12° and 15°C resulted in massive mortalities.

The purpose of this study was to determine the relationship between age, environmental condition, and otolith increment depositional rates in laboratory-raised striped bass larvae. This was accomplished by studying the increments of known-age larvae reared under both optimal laboratory conditions and restricted feeding regimes (laboratory-simulated suboptimal field conditions). Larvae were subjected to various periods of food deprivation to determine the potential dependence of increment depositional rates on nutritional condition. Specifically, incremental counts made with light microscopy and SEM were compared to evaluate the reality of apparent interruptions of daily deposition.

METHODS

Striped bass eggs were obtained from the Verplank Hatchery, Verplank, NY, within 24 hours of fertilization. Eggs were held in water obtained at the hatchery (0‰ salinity) at 18°C, under a 14L:10D photoperiod. Light levels were 25-31 μ Einsteins/m² per second. This light level is approximately equal to light at a depth of 2-3 m in a coastal stream or 1 m in a coastal estuary depending on turbidity and season of the year. Eggs hatched within 24 hours of fertilization. Newly hatched larvae were transferred to 4 L jars and stocked at densities of 50 per liter. Over the first 8 days, salinities were gradually raised to 5‰ by adding filtered seawater with 0‰ water. Seventy-six days after hatching of the larvae, salinities were gradually raised to 10‰ over a span of 8 days. Water was changed at least every other day.

Four feeding conditions were established. The food for all conditions was newly hatched brine shrimp, *Artemia*. Larvae were fed ad libitum (condition 1), other larvae were starved throughout the experiment (condition 2), and other larvae were starved for the first 15 days after hatching, then fed ad libitum (condition 3). Condition 4 consisted of larvae that were intermittently deprived of food. These larvae were not fed between 39-43, 51-55, and 62-66 days after hatching, for a total of 15 days out of the 68 days they were reared. For the remaining time they were fed ad libitum.

Larvae were sampled according to the schedule listed in Table 1. Larvae that were sampled were anesthetized with Tricaine methanesulfonate (Cresent Research Chemical[3]) and sacrificed. Total length was measured to the nearest 0.1 mm. Otoliths were teased from the otic capsules with fine dissecting needles, cleared of tissue, washed in deionized water and transferred with a micropipette or with fine dissecting needles to a labeled microscope slide.

Small otoliths were mounted permanently in *Euparol* without grinding. Larger otoliths were mounted in Flowtex (Lerner Laboratories), ground with 600 grit sandpaper and read with the light microscope. A subsample of ground otoliths was removed from the Flowtex, mounted in Spurr's medium, and ground to the core on Beuhler lapidary wheels. Initial grinding on the wheels began at 180

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[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

TABLE 1.—Sample size at age of striped bass larvae reared under four feeding regimes. N.T. = not taken.

Age (d)	Feeding regime condition			
	Continuously fed	Starved	Starved then fed	Intermittent starved
3	3			
4	8	5		
5	5	3		
6	5	5		
7	5	5		
8	5	5		
9	5	5		
10	5	5		
14	5	5		
18	5	5	2	2
22	5	5	2	2
26	5	6	2	2
33	5		2	2
38	4		N.T.	N.T.
40	2		3	2
47	5		2	2
54	5		2	2
58	1		N.T.	N.T.
64	N.T.		1	N.T.
68	3		3	2
97	5		2	

grit and final polishing was done with 0.25 μm diamond paste. These otoliths were etched with 0.02N HCl, then mounted on SEM stubs and sputter coated with gold/palladium.

Three light microscopes were used: a Zeiss, a Leitz, and an Olympus. The latter two were equipped with video viewing systems and polarized light sources. Readings were done with brightfield illumination at 400, 540, and 1,000 power. Video increased magnification to a maximum of 2700×. The maximum resolution for the light microscopes was 0.5-1.0 μm. The SEM employed was a JOEL (JSM 200) equipped with both secondary electron image (SEI) and backscattered electron image (BEI) collectors.

For light microscopy, slides were chosen at random and read double blind (age of the larvae and condition were unknown). Readings were done three times for each slide. Each slide was counted only once during each session so that replicate counts did not immediately follow each other. Thirteen of the twenty-four samples from conditions 3 and 4 were used for SEM analysis. For SEM examinations, counts were blind (ages of the larvae were unknown); condition, however, was selected by the investigators to check the accuracy of the light microscope counts for conditions 3 and 4.

RESULTS

Light Microscopy

The relationship between the number of otolith increments and age, in days, for the four experimental conditions is shown in Figure 1. Fully fed larvae ($n = 63$), condition 1, had a regression slope of 0.98 increments/day, and the smallest standard error (Table 2). Its confidence interval included 1 increment/day. Beyond 68 days of age sagittae became very difficult to read. Continuous counting paths or an appropriate series of transects were difficult to find because the sagitta changes shape and develops new centers of deposition around the periphery of the otolith. This resulted in underestimates of true age (Table 2) for larvae older than 2 months of age.
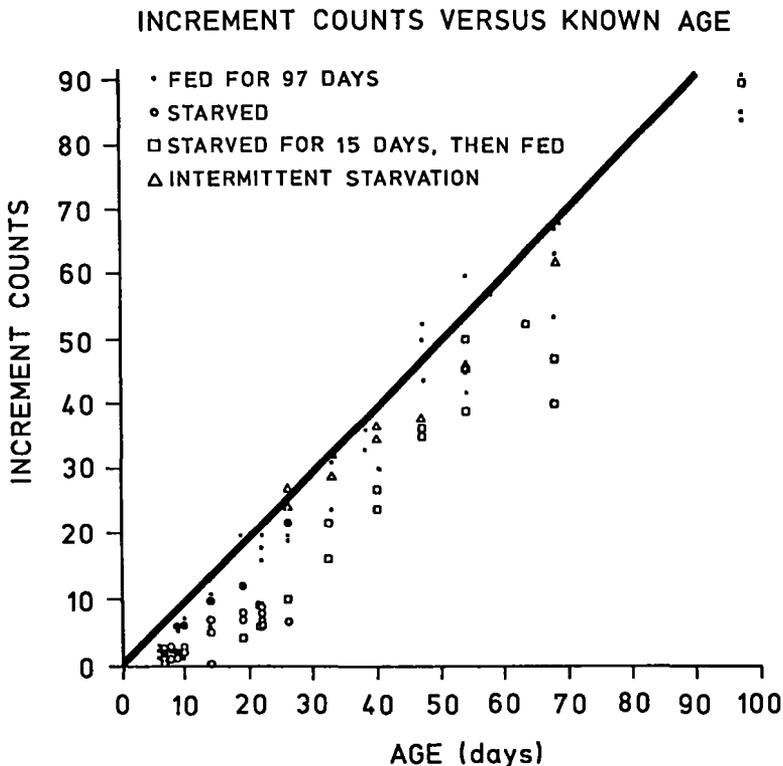

FIGURE 1.—Relationship between otolith increment count in larval striped bass and true age for four feeding regimes, light microscope observations.

TABLE 2.—Parameters for weighted regressions of increment counts on days from hatch of striped bass larvae reared under four feeding regimes. SE indicates standard error of the estimate, C.I. indicates confidence interval, N.S. indicates slope not significantly different than 1.0, * indicates P = 0.05.

Age	Condition	N	Slope		95% C.I.		Intercept		r^2	$P > 0.05$
			(counts/d)	SE (slope)	Low	High	Counts	SE (int)		
68 d and younger	1 Always fed	60	0.980	0.0243	0.931	1.029	−4.016	0.4482	0.96	N.S.
All ages	1 Always fed	63	0.946	0.0169	0.912	0.980	−3.627	0.4068	0.96	*
	2 Starved	43	0.469	0.0402	0.388	0.550	−1.697	0.4325	0.77	*
	3 Starved/fed	12	0.930	0.1005	0.711	1.149	−10.430	4.3906	0.90	N.S.
	4 Intermittent	12	0.873	0.0586	0.745	1.000	2.579	2.4010	0.96	N.S.

The slope of the regression line for starved larvae (condition 2, n = 43), 0.469 increments/day, differed significantly from 1.0 increment/day. Increments appeared regularly spaced. Otoliths of starved larvae did not appear aberrant under the light microscope.

The regression of increment counts versus true daily age for larvae, which were starved then fed (condition 3, n = 12), had a slope of 0.930 increments/day with confidence intervals which included 1.000 increments/day (Table 2). However, the regression intercept was − 10.430, an overestimate of age at first increment deposition. This leads to a 6-d underestimate of true age because depositional rates were underestimated during the first 2 weeks of life.

The slope of the regression line for intermittently starved larvae (condition 4, n = 12) was 0.873 increments/day. The slope of 1.0 increment/day fell at the very edge of the confidence interval. If a slightly smaller alpha level had been chosen, deposition would not have been assumed daily.

Initial increment formation began at 4 days after hatching with a 95% confidence interval that ranged from 3 to 5 days. Yolk-sac absorption occurs at 7 days after hatching at 18°C and first feeding begins at approximately the same time. However, initial increment deposition does not appear to be connected to these events. Two or three weakly defined increments were observed within the core in many SEM preparations. They were not counted in light or SEM readings.

Scanning Electron Microscopy

Results from the SEM study are qualitative rather than quantitative due to the small sample sizes, n = 13, used for the SEM. With SEM, otolith increment counts for condition 3, larvae which were starved then fed (Table 3), and for condition 4, larvae which were intermittently starved (Table 4), yielded more accurate counts than those obtained on the same specimens with light microscopy. With light microscopy counts from larvae which were starved for 15 days resulted in an underestimate of true age by 10 days (Table 3). The variability was also high (SE = 7.9 days). SEM counts underestimated true age by 2 days. Variability was small; the standard error was 3.4 and 4.0 days for SEI and

TABLE 3.—Counting bias for larvae starved for the first 15 days after hatch (calculated as estimated age − true age[1]). Underestimate of age is indicated by −; overestimate indicated by +. SEI = secondary electron image; BEI = backscattered electron image.

Sample no.	Age (d)	Microscopic technique		
		Light	SEI	BEI
1	68	− 17	0	+ 1
2	47	− 7	−5	−5
3	54	0	−5	−6
4	68	− 24	−5	−5
5	33	− 7	+4	+5
6	47	− 8	−2	−2
7	47	− 7	−1	0
Mean bias		− 10	−2	−2
SE		7.9	3.4	4.0

[1]Estimated age = number of increments + mean age at first increment formation.

TABLE 4.—Counting bias for larvae intermittently starved (calculated as estimated age − true age[1]). Underestimate of age is indicated by −; overestimate indicated by +. SEI = secondary electron image; BEI = backscattered image.

Sample no.	Age (d)	Microscopic technique		
		Light	SEI	BEI
8	68	+ 2	−5	−6
9	47	− 5	−5	−3
10	54	− 11	−1	−2
11	54	− 4	−4	0
12	47	+ 2	−4	−4
13	47	− 5	+1	+1
Mean bias		− 4	−3	−2
SE		4.9	2.4	2.6

[1]Estimated age = number of increments + mean age at first increment formation.

BEI, respectively. Light microscope-counted increments of intermittently starved larvae underestimated age by 4 days (Table 4). The standard error of the mean was 4.9 days. For this sample, both SEM techniques also gave more accurate age estimates. SEI underestimated age by 3 days (SE

= 2.4 days), and BEI underestimated age by 2 days (SE = 2.6 days).

The comparison between SEI and BEI showed no significant difference in accuracy (Tables 3, 4). Figures 2A and 2B illustrate the increment structure observed with these two methods of SEM. BEI
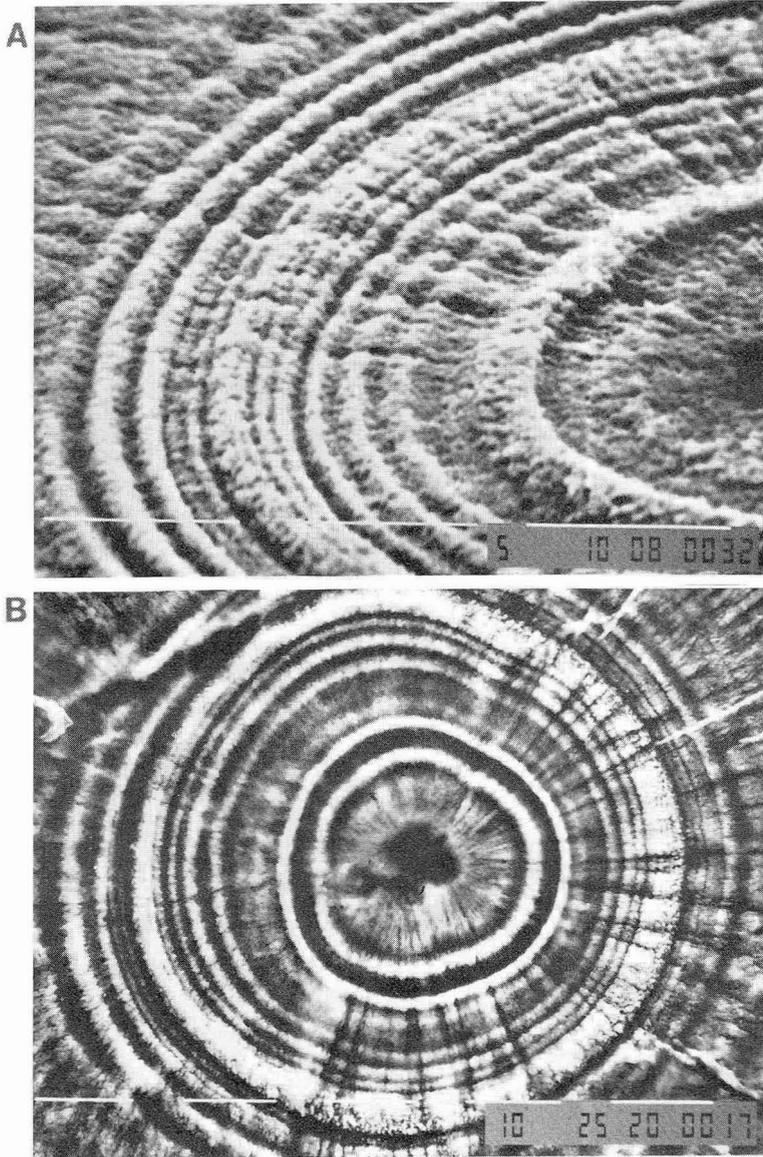
FIGURE 2.—Comparison of otoliths of larval striped bass using two scanning electron microscope (SEM), techniques. A) Normal secondary emission (SEI) photomicrograph of an otolith of a larva starved for the first 15 days posthatch then fed ad libitum until sacrificed. Increment width during starvation is approximately 5 μm. B) Backscattered emission (BEI) photomicrograph for a comparable otolith to A above. Legends in the micrographs indicate 1) length of scale bars in μm, 2) accelerating voltage KV, 3) mm working distance, 4) coded photo number.

enhances contrast, but does not allow the specimen to be tilted. With SEI, tilting can increase increment relief and visibility.

Increments deposited during starvation were only 0.5 μm in width, too closely spaced to be discerned with the light microscope (Fig. 3). Additionally, the material that is deposited appears to be more homogenous in density, probably containing a lower amount of matrix. When etched, less material was dissolved in the area corresponding to starvation periods. This resulted in a higher area of relief, forming a broad ridgelike structure, subdivided into finer increments. The etching properties were, therefore, different compared to the same area in the otolith of a fed larvae. This ridgelike structure consistently indicated periods of starvation during the first 2 weeks of life. Ridges were not apparent for older larvae starved for shorter time intervals (Fig. 4).

DISCUSSION

Estimation of age obtained using the light microscope was not always accurate. When larvae grew well, the light microscope gave correct age estimates. However, otoliths of larvae reared under suboptimal feeding conditions gave underestimates of true age. Age estimates were more accurate using SEM, and starvation episodes were easier to recognize in the otoliths.

Light microscopy has been routinely used to estimate age in field-captured larval fish (Jones 1985). Only a few investigators have employed SEM. Although SEM improves the accuracy of age estimates, it is more costly, requires more precise preparation, and is more time consuming. However, for larvae as resistant to starvation as striped bass, SEM verification of age estimation obtained with the light microscope is necessary. In our view, investigators using increments to estimate growth should check their results from the light microscope with SEM studies. SEM analysis could be performed on a randomly chosen subsample of otoliths. If problems were uncovered, a more extensive analysis using SEM could be undertaken. Checks on a random sample using SEM are particularly important for field studies where application of the otolith increment technique to estimate field growth is relatively new.

It could be argued that larvae which undergo periods of starvation are more vulnerable to predation and may occur only infrequently in samples. Although this is quite likely, it is precisely during
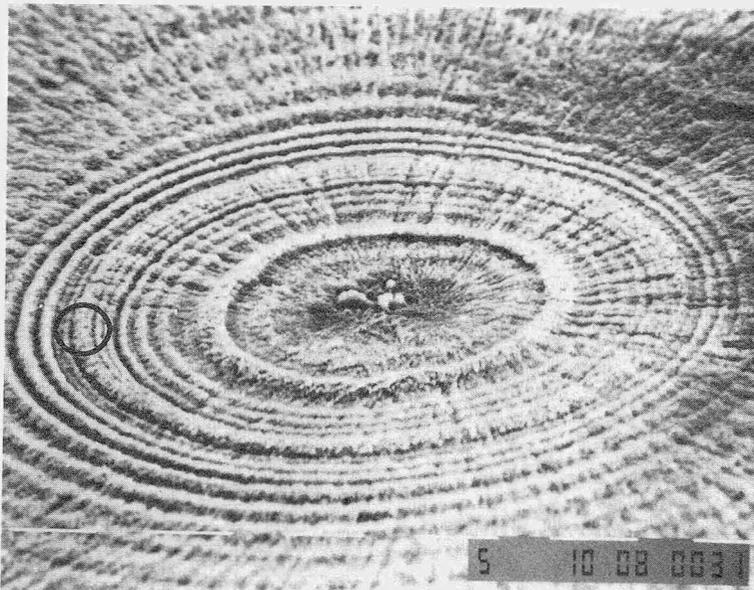

FIGURE 3.—SEM photomicrograph of an otolith of a larva starved for the first 15 days of life then fed ad libitum. A ridge, indicated by the circle, develops as the result of etching the otolith with 0.02 N HCl. This ridge corresponds to the period of starvation. Increment width during starvation is approximately 5 μm. Legends in the micrographs indicate 1) length of scale bars in μm, 2) accelerating voltage KV, 3) mm working distance, 4) coded photo number.
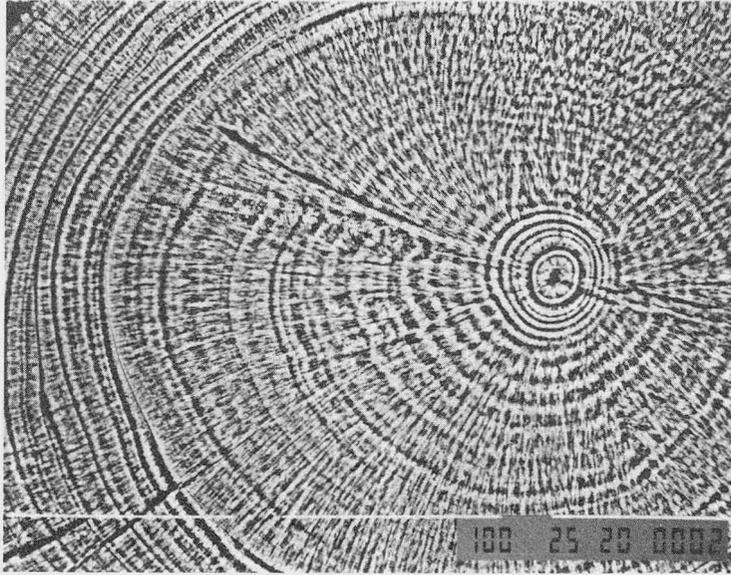
FIGURE 4.—SEM photomicrograph of an otolith of an intermittently starved larva. Note the pattern of narrow bands, indicated by the bracket, typical of starvation episodes. Increment width during starvation is approximately 5 μm. Legends in the micrographs indicate 1) length of scale bars in μm, 2) accelerating voltage KV, 3) mm working distance, 4) coded photo number.

years that have poor conditions, hence poor recruitment, that good age-based growth and mortality estimation would be the most useful. During such years, more of the young larvae could have their true age underestimated. This would result in overestimation of the abundance of younger larvae and therefore a steepened mortality curve. The best approach for routine field work may be to incorporate a design in which a small subsample of otoliths are analyzed by SEM to test for bias using the light microscope.

Bias in light microscope counts may account for the less-than-daily otolith increment deposition that has been demonstrated in the laboratory (Geffen 1982). For field-captured fish, there is no way of knowing whether light microscope counts are biased without the use of SEM. Additionally, this potential bias affects the variance of the estimate of size-at-age. When larval age is underestimated, under conditions which have resulted from poor growth, the variance is improperly decreased for young fish. Hence, lower variances may be a product of both high mortality and bias in age estimation.

Finally, the light microscope biases are more important for young larvae. By simple arithmetic, a bias of 3 days in a 7-d-old fish will result in a far more inflated growth rate than will a bias of 3 days (or for that matter 10 days) in a 60-d-old fish. The growth rate of the younger fish will be inflated 1.75 times compared with only 1.05 times (or 1.2 times using the 10-d bias) for the older fish.

ACKNOWLEDGMENTS

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