

Reprinted from "Journal of the Elisha Mitchell Scientific Society" Volume 116, 2000, pp. 251-259, Ahrenholz. Periodicity of growth increment formation in otoliths of juvenile gray snapper (*Lutjanus griseus*) and lane snapper (*Lutjanus synagris*). With permission from Dr. Frank J. Schwartz (Editor).

PERIODICITY OF GROWTH INCREMENT FORMATION
IN OTOLITHS OF JUVENILE GRAY SNAPPER
(*LUTJANUS GRISEUS*) AND LANE SNAPPER
(*LUTJANUS SYNAGRIS*)

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Abstract: The periodicity of growth increment formation in juvenile gray snapper (*Lutjanus griseus*) and lane snapper (*L. synagris*) sagittal and lapillar otoliths was determined with an otolith marking [alizarin complexone (ALC)] and captive rearing study. Resulting marks of low concentration ALC were located with green epifluorescent light and then confirmed with blue light. Increment formation rate for both types of otoliths and species did not statistically differ from one per day at the 95% significance level. However, in practical terms, the rate of increment formation was less than one per day for the lane snapper lapillar otoliths during the last two weeks of rearing (day 75 sample). Results from sagittal otoliths were more precise than those from lapillar otoliths.

Key Words: Gray snapper; *Lutjanus griseus*; lane snapper; *Lutjanus synagris*; otolith; growth increment; ageing; validation; verification.

INTRODUCTION

Gray snapper, *Lutjanus griseus* (Linnaeus) and lane snapper, *L. synagris* (Linnaeus) are tropical and warm temperate, marine reef fishes, found as adults from northern Florida to southeastern Brazil (Manooch and Matheson, 1981; Manooch and Mason, 1984). Juveniles of each species occur as far north along the U.S. Atlantic seaboard as Massachusetts. It is speculated that eggs and larvae of these fishes are carried north from their spawning grounds by the Gulf Stream (Adams, 1976). Juvenile gray snapper are commonly associated with lagoonal seagrass beds, and juvenile lane snappers co-occur in these and other inshore areas (Starck and Schroeder, 1971). Juveniles of both species inhabit the inshore sounds of North Carolina and can be locally abundant during summer and fall (Adams, 1976; pers. obs.), although the gray snapper appears to be the more common (Schwartz et al., 1981; Ross and Epperly, 1985). Ultimate fate of juveniles found north of their adult range is unknown, although in the absence of a documented estuarine emigration and southerly migration, it is speculated that they perish with colder winter temperatures (Starck and Schroeder, 1971). Contrary to this notion, adults of both species, although lane snapper predominate, have recently been caught on inshore reefs along the mid-North Carolina coast (Parker and Dixon, 1998), while both species have been taken for a number of years from inshore reef areas off Cape Fear, NC (although with gray snapper less common) (R. L. Dixon NOAA/CCFHR, pers. comm.).

Information on critical life history events for these snappers may be elucidated through analysis of otolith microstructure. Analyses that utilize daily ages and spawned-date distributions to determine growth rates and recruitment success depend on the accuracy and precision of presumed periodicity of (daily) increment formation. This study determined periodicity of increment formation in otoliths of juvenile gray and lane snapper during late summer and early fall of 1993 and examined, quantitatively and qualitatively, the efficacy of the lapillus and sagitta for microstructural analyses.

MATERIALS AND METHODS

Juvenile snappers were collected during the week of 2 August 1993 from submerged seagrass beds in eastern Core Sound, North Carolina. Juveniles were captured with a 2 m otter trawl (6 mm bar body, 3 mm bar tail bag) deployed from an outboard-powered, sixteen-foot skiff. Specimens were transported to the NMFS Beaufort Laboratory and maintained in a fiberglass tank with flow-through, ambient seawater. Juveniles were immersed 6 August in 30 l of 100 mg L⁻¹ alizarin complexone (ALC) solution for 15 hr. The solution temperature was 26.5°C on 6 August, but because of a problem with the temperature control, the temperature dropped to 21.5°C by the following morning. Thirty-two gray snapper and 14 lane snapper were released 7 August into a 6,600 L concrete rearing pond, equipped with a flow-through seawater system. The snappers were fed chopped shrimp, fish, and soft crab at least once daily for the duration of the study. Two to three individuals of each species were sampled about every two weeks and preserved in 95% ethanol.

Sampled fishes were weighed, measured (total length), and sagittal and lapillar otoliths removed by dissection and cleaned in a 5% sodium hypochlorite solution. Otoliths were rinsed in distilled water, placed on pre-cured droplets of epoxy resin, dried, then embedded in freshly mixed resin. Each embedded otolith was mounted on a slide etched with parallel lines for orientation and cross-sectioned with a pair of diamond wafering blades spaced 0.5 mm apart (Fig. 1). Resulting sections were ground and polished to 25–50 µm thickness.

Prepared otolith sections were examined using a compound microscope (40 and 100×) and on an adjoining image analysis system monitor (1,520 and 3,800×). The ALC-marked increment on each otolith section was located with both blue and green light epifluorescence. All the otoliths were read on the video monitor at 1,520× except for the gray snapper lapilli, which were read at 3,800×. Post-mark increments were tallied with a hand held counter. Means of four independent counts (one reader) represented the estimate of the number of increments from the ALC mark to the margin. The image analysis system was also used to measure growth increment widths for periods before and after marking.

Statistical procedures were similar to those reported by Ahrenholz et al. (1995). Analyses consisted of first performing linear, least squares regression analysis (SAS Institute, 1985) of post-mark increment counts on rearing duration in days. Student's *t* tests were then performed to test the significance of the regression parameters. The basic validation null hypotheses tested (95% level) were that the intercept was not significantly different from zero, and that the slope was not significantly different from one. Additionally, statistical power was calculated to detect real differences in the slope estimates (Rice, 1987). Finally, 95% confidence

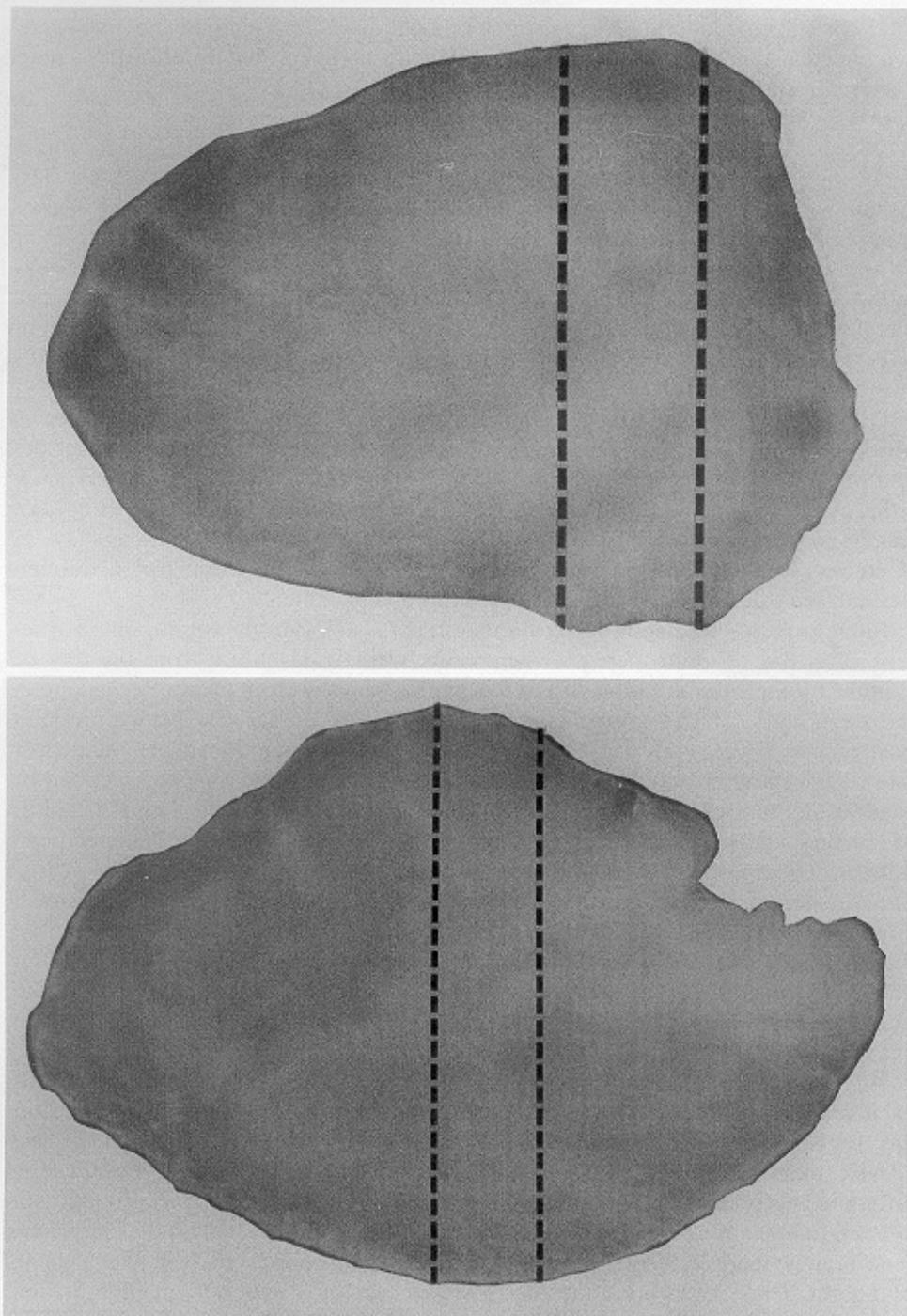


FIG. 1. a) Proximal side of the left lapillus from a 79 mm TL lane snapper (*L. synagris*). Orientation of sectioning is shown with dashed lines. The dorsal-ventral height of the otolith (section) is 0.754 mm. (Orientation of the otolith is as follows: Dorsal-top and anterior-right.)

b) Proximal side of the left sagitta from a 51 mm TL lane snapper. Orientation of sectioning is shown with dashed lines. Dorsal-ventral height of otolith (section) is 1.823 mm. (Otolith is oriented as follows: Dorsal-top and anterior-right.)

intervals (CI) for individual age estimations obtained from increment counts were calculated using the *inverse prediction* condition (Sokal and Rohlf, 1981; note discussion in Rice, 1987).

RESULTS

At capture, both species of juveniles ranged in size from 2.5 to 4.5 cm TL, although the lane snapper were dominated by larger individuals and the gray snapper by smaller individuals. Unfortunately, because of predation resulting from an accidental introduction of a large pigfish, *Orthopristis chrysoptera* (Linnaeus) at the outset of the rearing portion of this study, only 10 lane and 8 gray snapper were available for analyses. Two lane snapper were sampled on post-mark rearing days 19, 33, 48, 61, and 75, while three gray snapper were sampled on day 19, two on days 33 and 48, and one on day 61.

Growth rates appeared to be relatively slow, as compared to the same species observed in the field (e.g., a sample of six juvenile gray snappers taken at the end of September 1996 ranged from 64 to 140 mm TL). The gray snappers grew from about 36 mm on day 19 to about 55 mm on day 61 (October 6) (Fig. 2a). Lane snappers increased from about 62 mm on day 19 to 96 mm on day 75 (October 20). Pond water temperatures were relatively stable about 26.5°C through day 30, then declined to near 22°C by day 70 (Fig. 2b).

Slow growth was also reflected in increment spacing in the otolith microstructure (Fig. 3a). Prior to capture, increments were wide, while from the day of capture through the duration of the study, the bands were narrow. For example, the mean width of 10 increments measured along the dorsal sulcal ridge pre- and post marking were 4.6 and 2.8 μm for the lane snapper. Mean pre- and post marking increment width along the dorsal lobe of the gray snapper sections of sagittal otoliths were 10.3 and 3.2 μm . Similar patterns resulted with the lapilli. Mean pre- and post marking increment widths for lane and gray snapper were 4.9 and 2.6 μm , and 5.4 and 2.3 μm , respectively.

Estimates of rate of growth during the study were obtained as the slope of a simple linear regression of the mean size at time of sampling. Mean growth rate for the gray snapper was 0.36 mm day⁻¹ and for the lane snappers was 0.70 mm day⁻¹. When growth rate is expressed as a percentage of mean total length, the rates for the two species are almost equal (0.8 for gray snapper and 0.9 for lane snapper).

At least one lapillus and one sagitta were successfully processed and read for each reared specimen, except for one lapillus from a lane snapper sampled on day 19. Thus, two sets of validating increment counts were made for each species.

ALC marks on the otoliths were difficult to detect with blue light. Fortunately, a fluorescing pink mark was detectable against the resulting red background with a green light source (Fig. 3b). This mark was more readily seen than the diagnostic pink-orange mark against the resulting green background obtained when using blue light. Thus, by locating the ALC-marked increment with the green light, and then studying that site under the blue light (without adjusting the focus), the verifying shades of diagnostic pink-orange were eventually detected.

Statistical analyses revealed that the validating criteria for daily periodicity of increment formation were met, i.e., the null hypotheses tested could not be rejected (Table 1). However, the results for the lapillar readings for lane snapper

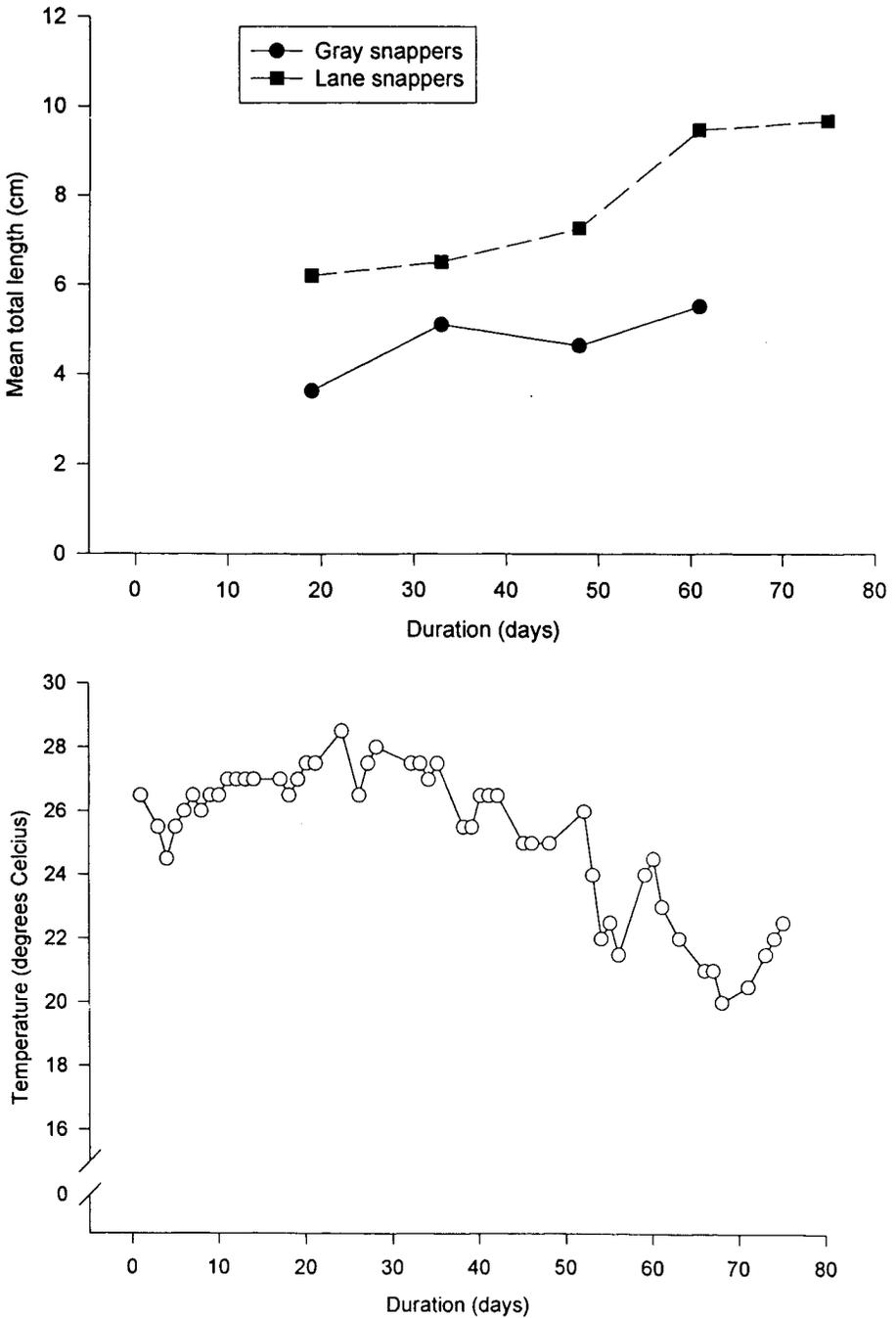


FIG. 2. a) Mean total length for ALC marked and reared gray (*L. griseus*) and lane (*L. synagris*) snappers.

b) Ambient rearing water temperatures for ALC marking experiment for gray (*L. griseus*) and lane (*L. synagris*) snappers.

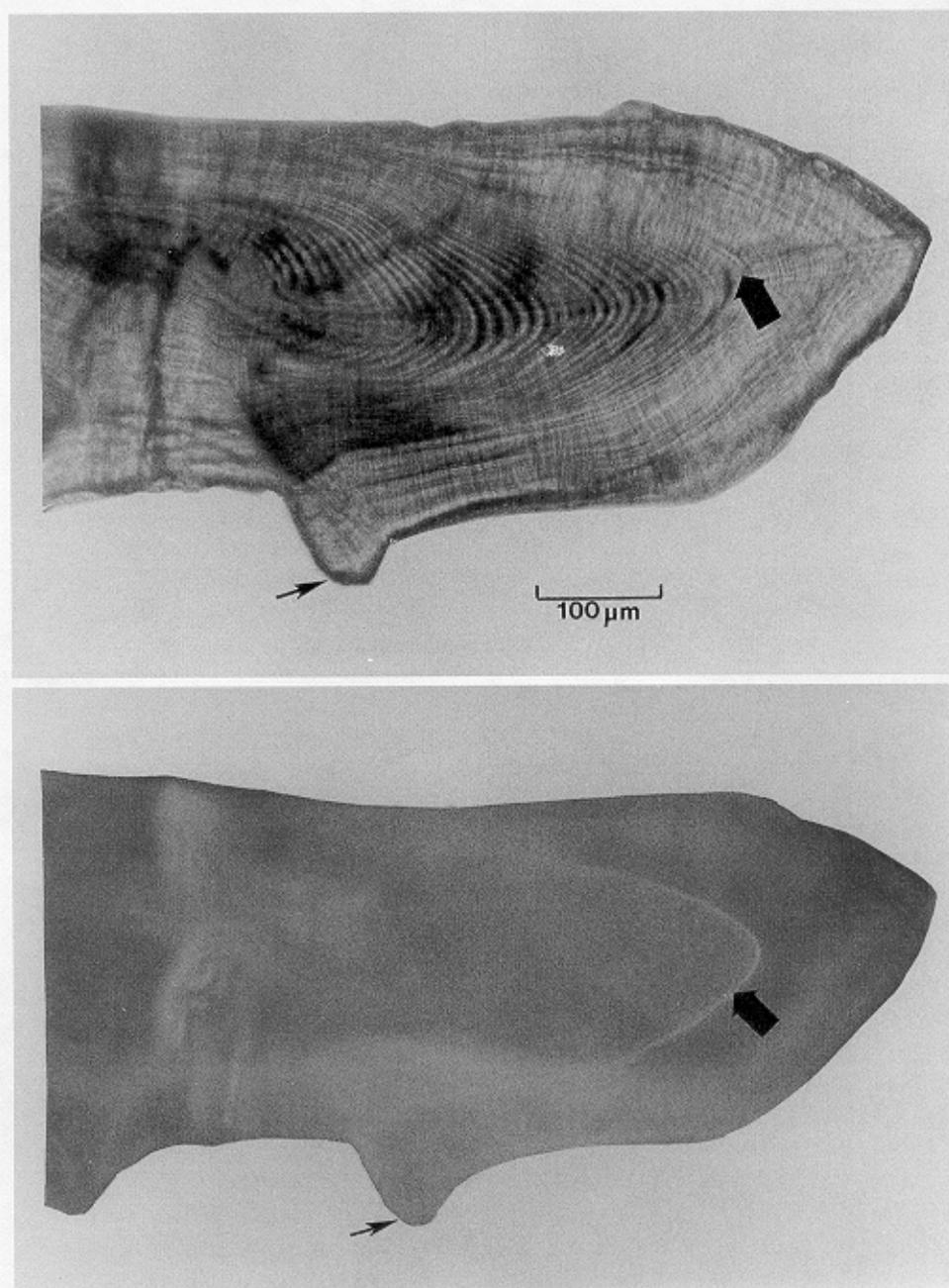


FIG. 3. a) Photo-micrograph (white light) of the dorsal portion of a cross-section of a sagittal otolith from a 55 mm TL gray snapper (*L. griseus*) sacrificed 61 days following ALC marking. Note wide increments prior to approximate point of field capture (thick arrow) and narrow increments during the experimental captive rearing period. Thin arrow denotes the dorsal ridge of the sulcus.

Table 1. Results from least squares linear regression analyses of increment counts for alizarine-complexone marked otoliths from juvenile gray snapper (*Lutjanus griseus*) and lane snapper (*L. synagris*). The null hypotheses tested are that the slope = 1 and the intercept = 0.

Parameters	Gray Snapper		Lane Snapper		
	Sagitta	Lapillus	Sagitta	Lapillus	Lapillus ^a
n	8	8	10	9	7
r ²	0.997	0.975	0.997	0.956	0.987
Intercept	-0.724	1.127	-0.221	5.290	-0.447
SE	0.784	2.165	0.865	3.454	2.201
P	0.391	0.621	0.805	0.169	0.847
Slope	1.005	0.935	0.987	0.855	1.011
SE	0.021	0.057	0.017	0.064	0.048
P	0.830	0.295	0.453	0.059	0.826
% Power ^b	0.981	0.329	0.999	0.280	0.418
95% CI ^c	3	8	3	11	6

^a The two 75 day duration data points are omitted from this analysis.

^b The statistical power to detect a difference of 0.1 from a slope of 1 at the 95% level.

^c The number of \pm days required to calculate the 95% CI at 61 days post mark for an *inverse prediction* (Sokal and Rohlf, 1981).

were marginal. Readings for the two 75-day lapilli (last rearing period) fell short of daily formation, at least in practical terms (mean counts of 65.0 and 66.5). Hence, these last two data points were omitted and an additional analysis performed for comparative purposes (Table 1, last column).

DISCUSSION

Juvenile gray and lane snappers from a North Carolina sound support the expectation that daily periodicity for growth increment formation in sagittal otoliths occurs during late summer and fall. Although the experimental evidence was not as strong, the results also confirm daily periodicity for growth increments on lapillar otoliths of both snapper species. Admittedly, rearing conditions were less than ideal and may have caused reduced growth rates (less than field levels). Thus, less than daily periodicity of growth increments would be plausible. Szedlmayer (1998) noted a less than daily increment formation rate in sagitta from captive juvenile red snapper, *L. campechanus* (Poey) when growth rate was below 0.3 mm day⁻¹.

ALC marks on experimental otoliths were difficult to discern when using the conventional blue-light epifluorescent light, but were resolved by first using green light. The faintness of the mark was most likely caused by a low concentration of ALC in the marked increment. Reasons for the low concentration may be: 1) the strength of the ALC immersion solution was too low relative to the specimen size, and/or 2) the accidental lowering of the water temperature during the mark-

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b) Photo-micrograph (green-light epifluorescence) of the same gray snapper otolith section shown above with fluorescing ALC mark (thick arrow). The ALC mark is pink against a red-pink and black back ground. Thin arrow denotes the dorsal ridge of the sulcus. (See text for discussion of blue-light epifluorescence with ALC.)

ing process reduced metabolic rates and thus decreased the amount of ALC absorbed.

Overall, the sagittal results displayed more predictive strength and a higher level of precision (narrower 95% CI) than those for the lapilli (Table 1). At least some of the reduced efficacy for the lapilli may be a result of the orientation of the sectioned otolith. Given the orientation of growth increments and their narrow width, counts on prepared lapillar sections were only possible near the dorsal/distal tip. Conversely, broad regions of sectioned otolith surfaces with good contrast were readily found on sagittae of both species. Sagittal otolith sections of lane snapper were generally read near the ventral sulcal ridge, while similar sections for gray snapper were read more ventrally. Moreover, no two sagittal otoliths were read in precisely the same region. Perhaps lapilli ground on a sagittal plane, as performed by Brothers and McFarland (1981) for lapilli from French grunt, *Haemulon flavolineatum* (Desmarest), would potentially provide a greater number of counting paths.

Lower than expected counts for the day 75 lane snapper lapilli do not appear to be a condition limited by the resolving threshold of light microscopes as reported by Morales-Nin and Ralston (1990) for taape, *Lutjanus kasmira* (Forsskål). Increments near the tip of the lapillus for each of the two specimens were between 1.5 and 2.0 μm width. Measured widths were much greater than the theoretical limit of 0.2 μm reported by Campana and Neilson (1985) or the practical limit of 0.8 μm as defined by Morales-Nin and Ralston (1990).

My findings and procedures should support and foster more extensive studies that examine growth rates and spawned-dates of gray and lane snappers in the U.S. South Atlantic coastal waters. These factors can differentially impact the survival and subsequent fishery recruitment of juveniles of these two species of snapper along the north-south gradient of their nursery range. Additional studies that incorporate tagging and/or otolith growth patterns as revealed by examinations of otolith microstructure may provide a linkage between geographic nursery sites and adult populations.

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