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Validation of Black Sea bass, *Centropristis striata*, Ages Using Oxytetracycline Marking and Scale Margin Increments

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Abstract

Black sea bass age determination methods, as well as timing and periodicity of annulus formation were examined using scales and otoliths collected from the US commercial fishery and research vessel bottom trawl survey cruises. Edge analysis of 714 scale samples collected during 2011 was conducted. We examined both otolith and scale structures from 55 individuals marked with oxytetracycline sacrificed at regular intervals over two year period. Results indicated that annulus formation takes place annually during the summer (July-September) and that no other marks appeared on the scales or otoliths of marked individuals during the study period. A side-by-side comparison of precision and bias was performed between scales and otoliths, as well as, between laboratories. We concluded that scales and otoliths were of equal utility ageing black sea bass. There was good agreement between labs (82%); however the Bowker's test showed there was a bias. The bias was due to the disagreements between age 0 and age 1. Specific resolutions were established to clarify the bias; including processing methodologies and various calibrations to known ages. These validation and precision comparison results demonstrate that black sea bass ages from scales and otoliths from both laboratories can be used in the assessment.

Introduction

The black sea bass, *Centropristis striata* (Linnaeus), is a protogynously hermaphroditic serranid found off the east coast of the United States. This important commercial and recreational species is widely distributed on the continental shelf from Nantucket Sound, MA, southward to the Florida Keys (Miller 1959; Fisher 1978) with a maximum abundance between New Jersey and North Carolina (Kendall and Mercer 1982). Based upon several criteria including tagging studies, seasonal distribution, differences in time of spawning, and growth rates, Mercer (1978) defined black sea bass, north and south of Cape Hatteras , NC, as two separate stocks. The southern stock occupies coral and sponge reefs (Struhsaker 1969; Powles and Barans 1980) and does not appear to undergo seasonal migrations. Black sea bass north of

Cape Hatteras inhabit coastal waters of the Middle Atlantic and southern New England regions during their spawning period from late spring to early autumn, but migrate to deeper waters along the continental slope for the winter months (Musick and Mercer 1977). Tag returns also suggested that individuals north of Cape Hatteras constitute a unit stock (Kolek 1990), and a study of meristic and morphometric characters indicated phenotypic variations occur latitudinally (Shepherd 1991). Analytical assessments of the northern stock of black sea bass have been hindered in part due to the lack of confidence on the age composition of the landings and population.

The first step in evaluating the age composition of a stock is to verify the accuracy and precision of the ageing method. Numerous studies examining the biology of black sea bass have used a variety of structures to age this species (Lavenda 1949; Dias 1971; Cupka et al. 1973; Briggs 1978; Mercer 1978; Alexander 1981; Low 1981; Link 1984; and Wenner et al. 1986). Lavenda (1949) aged black sea bass collected off New Jersey from cellulose acetate impressions of scales and described annuli as "bunching of circuli" assumed to form during winter. This method was not validated however, and subsequent studies suggested that Lavenda's ages were overestimated (Dias 1971; Mercer 1978; Link 1984). The only study to attempt a validation of ages derived from examination of black sea bass scales was conducted by Briggs (1978) using marginal increment analysis and back-calculation. However, the results of this study had limitations since sample size was small and the coverage area did not represent the entire northern stock. If black sea bass ages derived from scales are to be used, to conduct proper analytical assessments, accuracy of those ages needs to be ascertained by collecting samples that cover the entire northern stock.

Otoliths have been used to age black sea bass from below Cape Hatteras (Cupka et al. 1973; Wenner et al. 1986), areas north and south of Cape Hatteras (Mercer 1978), and southern New England waters off of Long Island, NY (Alexander 1981). There have been various validation studies on black sea bass otoliths. Cupka et al. (1973) used otoliths from fishes collected off South Carolina and used back-calculation methods to validate his methods. Mercer (1978) examined otoliths from fish collected in the mid-Atlantic bight and used back-calculation, marginal increment and length-frequency modal analyses to validate ages. However both these studies only sampled southern and mid-Atlantic bight and due to the differences in growth patterns, might not representative of the northern stock.

Dias (1971) found otoliths to be superior to scales, vertebrae, and subopercular bones for black sea bass in Virginia waters. Dery (1985) indicated that examination of the sections of pelvic spine and scales were acceptable alternatives to otoliths as ageing structures based on evaluation of average percent error (Beamish and Fournier 1981) values calculated between structures. Due to the different migration patterns and inhabitation displayed by the north stock, Black sea bass could have clearer marks deposited onto the scales compared to their southern counterparts. If that is the case, scale age estimates could be as precise as otoliths for northern stock black sea bass.

Age structures (scales or otoliths) were collected from the US commercial fishery by the National Marine Fisheries Service (NMFS), Northeast Fisheries Science Center (NEFSC) since 1980. The objective of this study was to validate age determination methods of black sea bass from the northern stock. To accomplish this, we used reference marks in both scales and otoliths formed by injections of oxytetracycline in laboratory-held fish, edge analysis and marginal

increment analysis from samples collected from the commercial fishery and surveys to verify the timing and periodicity of annulus formation. Once accuracy and precision of both hard-parts are ascertained, we compared precision between hardparts and laboratories.

Materials and Methods

Edge Analysis

Edge analysis was performed on 714 scale samples collected monthly during January to December 2011 from the US commercial fishery (Table 1). All samples were comprised of black sea bass collected from waters north of Cape Hatteras, NC to Nantucket Sound (Figure 1). Scale samples were impressed on plastic laminate (Dery 1983) and viewed with a microprojector at 20x or 50x magnification. A combination of the aging criteria described by Lanvenda (1949) and Dery and Mayo (1988) were used to determine annuli, which they defined as "areas of closely spaced circuli with cutting and crossing-over marks, forming dark bands about the scale." To examine annulus formation, presence or absence of annulus on the edge of the scale were made on each sample. Monthly percentage of presence/absence were plotted to determine timing and periodicity of annulus formation (Figure 2).

Timing and Periodicity of Annulus Formation

During May and June, 1990, 55 live black sea bass (25-43 cm total length, TL) were obtained from commercial fishermen and were transported to the NEFSC, Woods Hole Laboratory. Fishes were held in five 200-gallon aerated tanks filled with ambient water from the adjacent Great Harbor, Woods Hole, MA. Daily water temperatures and photoperiods were monitored and adjusted to simulate seasonal environmental changes. Individuals were fed to satiation three times a week on a diet of butterfish, herring, squid and clams.

After a one month acclimation period, experiments were performed on 16 individuals to determine appropriate methods of injection and dosages of oxytetracycline following procedures described by Weber and Ridgway (1962) and McFarlane and Beamish (1987). Individual black sea bass were separated into four groups, anesthetized in a 1.5 mg methylmodate/l sea water solution, measured (TL, mm), weighed (0.1 gm), injected with varying doses of oxytetracycline and tagged (Floy t-anchor type). Experimental dosages for the four groups were 15, 25, 50, and 75 mg oxytetracycline/kg of total body weight. In each group, two fish were given intraperitoneal and two given intermuscular injections.

Fourteen days after the injections, one individual from each injection-type and dosage group was sacrificed and scales and sagittal otoliths excised. Each structure was viewed at 2x magnification under epifluorescent ultraviolet light at 405 nm wavelength, 455 nm dichroic mirror, and 455 nm barrier filter. We determined that intermuscular injections at 50 mg/kg dosages, were the most effective for this species, providing clear, well defined fluorescent bands on the scales and otoliths. All remaining black sea bass (n = 39) were then tagged and injected inter-muscularly with 50 mg/kg dosage and placed in the holding tanks. Individuals were randomly selected and sacrificed at one to two months intervals for two years (Figure 3 & 4). To examine incremental growth and annulus formation, measurements (mm) from the last annulus to the edge of the scale and otolith were made on each sacrificed fish. Mean marginal increments were calculated in each monthly sample and mean monthly increments plotted to determine timing and periodicity of annulus formation (Figure 5).

Measures of Precision

A comparison of calcified ageing structures was conducted between the Northeast Fisheries Science Center (NEFSC) and the Virginia Institute of Marine Science (VIMS), two organizations that routinely age black sea bass samples from northern stock. The NEFSC supplied 200 paired otolith samples for this comparison. A single whole otolith from each pair was retained by the NEFSC and read whole following the Center's protocols. Specifically these whole otoliths were cleaned to remove sacculous membrane and read under a stereo dissecting microscope using reflected light against a black background in a petri dish containing water. Magnification ranged from a 12x zoom objective lens to a 50x zoom objective lens. The second otoliths of each pair were sent to VIMS to be sectioned under their current processing methodology and protocols.

At VIMS, the whole otoliths were first mounted to a thin square of Rite-in-the-Rain brand paper using a clear pliable bonding agent called Crystal Bond. The Crystal Bond is heated to 70 degrees Celsius in a Pyrex dish on a hot plate to create a viscous liquid state. The Crystal Bond will cool at room temperature and become a solid again within 30 seconds. The small paper squares used to mount the otoliths have a double line thin cross printed on them to properly position the otolith for sectioning. The otolith is positioned with the double vertical lines on the paper perpendicular to the succal groove while passing directly through the nucleus with the succal grove side of the otolith mounted down. The positioning takes place over a 10-15 second span while the Crystal Bond is hardening. A thin transverse section was cut through the otolith's nucleus, or core, using two diamond wafering blades on a Buehler IsoMet slow-speed saw lined up with the printed cross on the paper square. The two blades are spaced using three honed sections of old wafering blades that are 0.18mm thick. The transverse section was then cut to a 0.54mm thickness. The section was then mounted to a glass slide with Crystal Bond. The assigned number was written on the slide once the section was mounted. The sections were then polished briefly using 400-800 fine grit sandpaper and again covered over with Crystal Bond to assure the clarity in the section.

The samples were read under a stereo dissecting microscope with transmitted light using magnification of 25x zoom objective lens, which is preferred for standardization. 12x and 50x objectives were used occasionally depending on the size of the section and the number of visible annuli. Confidence of each read for a specimen was recorded. Confidence assignments ranged from 5 to 1, 5 being most confident and 1 being least confident. Factors that coincided with confidence were light, opaqueness, deformations in the crystalline calcium structure and quality of the processed section.

To measure in between laboratories precision, a subsample of 200 fish representing both spring and fall of 2012 and 2013 Bottom Trawl Surveys. Between and within reader precision was measured using Chang's (1982) average coefficient of variation (CV) with the formula presented in Campana and Jones (1992):

$$100\% * \sqrt{\frac{\sum_{i=1}^{R} \frac{(X_{ij} - \overline{X}_j)^2}{R-1}}{\overline{X}_j}}$$

Equation 1. Calculation for Chang's coefficient of variation.

where X_{ij} is the ith age determination of the jth fish, X_j is the mean age of the jth fish, and R is the number of times each fish is aged.

Measures of bias

A Bowker's test (Hoenig et al. 1995, Bowker 1948) was used to test for departures from symmetry within the age-frequency table. Such asymmetries indicate the presence of a bias, although the test has low sensitivity when few disagreements exist. Where ages differ from one another, the Bowker's test compares values on the age-frequency table which represent symmetric errors, such as the paired ages (3,4) and (4,3). If all such values are dissimilar, the test will return a significant P value. This test statistic is calculated as a chi-square variable, as follows:

$$X^2 = \sum_{i=1}^{m-1} \sum_{j=i+1}^{m} \frac{(n_{ij} - n_{ji})^2}{n_{ij} + n_{ji}}$$

Equation 2. Calculation for Bowker's test of symmetry.

where m is the maximum age in the data set, and n_{ij} is the number of fish in the ith row and jth column (Hoenig et al. 1995, Bowker 1948). The value of the degrees of freedom is equal to the number of non-zero n_{ij}-n_{ji} comparisons in this calculation, to a maximum of m(m-1)/2.

Results

We aged and performed edge analysis on 714 black sea bass. Ages ranged from 2 to 5; reflecting the dominance of these age classes in the commercial landings. Percent presence of annuli on the edge for all ages had the same general pattern. Percent presence of annuli on the edge increased from about July to September, declined precipitously in October, and no annuli on the edge was found from November through June. These results suggest the black sea bass scales annulus formation took place during the summer months (July-September). Circuli spacing was relatively constant and there were no false annuli or spawning checks evident on the scales collected during the study period, indicating that annuli are formed annually for age groups 2-5.

Black sea bass injected with oxytetracycline had clear, compact, well defined fluorescent bands on scales and otoliths. This indicated that the tetracycline was incorporated within 24-48 hours of injection on only the newly developing edges of the structures (Figure 3 and Figure 4).

Measurements and age determinations made on captive individuals marked with oxytetracycline showed the same annual pattern on their scales as the samples collected from wild fish. The age of these fish ranged from about 2-5, similar to the age composition of the commercial fishery. Injections were made at the time of annulus formation in June 1990 and the fish were sacrificed at one to two months intervals through two full growing seasons. It was not possible to track the growth or age of individual fish because all tags placed on the black sea bass at the time of injection were lost over the course of the study period. Fishes "removed" tags by biting and pulling them from others.

The laboratory study showed the same pattern of growth on the scales and otoliths in both years: increasing marginal increments from about September to May and minimal edge deposition from July to August. This corresponded exactly to the marginal increment pattern described for wild fish (Figure 5). Sacrificed individuals ranged from age 2-4 (4% age 2, 25% age 3, and 71% age 4) in 1991 and were age 4 (87%) and 5 (13%) in 1992.

Precision at the NEFSC using scales was quite high with a 90.6% agreement and a CV of 1.60%. Agreement for otoliths was a little lower with 88.9% agreement and a CV of 2.09%. Between scales and otoliths agreement was 75.3% agreement with a CV of 4.55%. None of the tests showed any bias. The VIMS sectioned otolith samples were independently read twice by each senior ageing personnel. Each read was temporally separated by two weeks to promote independence of these reads. After the VIMS first read, there was slight over-ageing occurring from the Reader 1 reads. Overall agreement was 77.6% with a high CV of 16.14%. Corrections were noted after the first round reads and second round read agreement had significant improvement. Agreement for the second read was 97.4% with a CV of 1.02%. Neither of the tests for the reads showed any bias. Ages from the second read were used as the VIMS final ages. Consensus ages were reached for the few samples that were still in disagreement after the reads and tests of symmetry were conducted. Agreement between labs (Figure 7) using otoliths was good with an 82.1% agreement. However, the CV was high with 8.88% and there was systematic bias ($p=0.04$).

Discussion

Our study validated a method commonly used to age *C. striata*. Marks on scales and sagittal otoliths occurred annually, with no other visible marks. Annulus formation takes place once annually during the summer corroborating the results of Briggs (1978) and Dery (1985), either as minimized spacing of circuli, "bunching" with clear crossing over marks on the lateral sides of the scales, or the formation of hyaline zones on otoliths (Lavenda 1949; Dery and Mayo 1988). This is probably the result of minimized somatic growth and a transfer of energy to the reproductive process during this period. Oxytetracycline injections confirmed that sagittal otoliths and scales were both suitable structures for ageing this species. This is important since both methods are currently used, but from the standpoint of non-lethal and efficient collection and sample processing, scales would be the preferred ageing structure to use.

The importance of validating the accuracy of age determinations and attempting to validate all of the ages found in a population (Beamish and McFarlane 1983) cannot be overstated. Important biological reference points used currently in the management of fisheries depend upon accurate age data. Yield and spawning stock biomass per recruit models, and cohort

based analytical assessments are grounded on basic age data whether in the form of growth curve parameters, estimated mean size at age, or maturity at age.

The precision comparison between the NEFSC and VIMS of the 200 paired whole and sectioned otoliths was close to passing the 80% agreement standard set by the NEFSC Fishery Biology Program ageing group. The initial study compared the paired otoliths collected by the NEFSC and processed and read by the two agencies to their preferred methodology of ageing; NEFSC read whole otoliths while VIMS read transverse sections of the opposite pair. The overall agreement was 79.1% with a 9.96% CV and a p-value of 0.02, displaying some bias between the agencies (Figure 6). The error bars represent 95% confidence intervals. The bias derived from an age 0 and age 1 discrepancy. Despite the overall average passing minimum criteria, the two agencies agreed to take a further look into those discrepancies. Pictures of the samples in which there was a disagreement were taken by each agency to confirm their final ages.

After a period of time the precision comparison was revisited and the samples with the disagreements were reread by both agencies. The adjusted overall agreement after the rereads was slightly improved to 82.1% with an 8.8% CV and 0.04 p-value (Figure 7). The final ages from the second reread showed there was still a bias between the labs for ages 0. The age 0 sample bias from the original reads and the rereads is a byproduct of the clarity of the first annulus on sectioned otoliths versus the whole otoliths. The first annulus often forms very tight to the nucleus of the otolith, thus making first annulus location and assignment difficult. The bias resulted in samples under-aged by the NEFSC and overaged by VIMS. This bias only occurred for fish smaller than 15cms collected in the fall. Therefore we recommend that the stock assessment use only fish greater than 15cms in the fall.

Analysis of overall reader agreement spanning across several black sea bass workshops, exchanges and comparisons there has often been shows a peer-influenced bias resulting in higher agreement between readers and between across agencies at these juxtaposed workshops. In addition to the black sea bass workshops, this observation has been acknowledged with additional species workshops and exchanges. The improved (and almost perfect) agreement at these workshops is a result of verbal and physical bias. The proximity of additional readers often improved confidence of the readers, as well as influenced agreement, which resulted in a marginally improved overall agreement of the group at the workshop, opposed to the original independent reads. Additionally, readers could tend to drift from their calibrated reads over time. The drift should be considered a bias and should be accounted for by conducting calibration reads from a species specific sets of otoliths by that species with known established ages. Both VIMS and the NEFSC conduct calibration reads when it is applicable for final age assignment of a species. It should be noted that overall agreement for the two agencies passed necessary criteria to be declared acceptable for assessments. Further work between the two laboratories will continue to improve overall agreement for black sea bass as well as other managed species for various assessments.

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Table 1. Numbers of black sea bass collected in 2011 by size and by month for edge analysis.

Size (cm)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
25			1					1					2
26	1		2		1		1	2					7
27			5		2	1	3	3		2	1	3	20
28	2	3	4		6	1	4	5	5	6	10	7	53
29	4	4	4		6	4		6	5	5	11	2	51
30	10	2	4		3	3	3	10	6		4		45
31	9	5	4		5	1	1	4	6	2	2	3	42
32	10	2	4		5	1	5	5	3	5	13	1	54
33	7	2	5		7	5	9	2	5	1	9	3	55
34	6	2	5		14	5	12	3	1	6	5	3	62
35	1	1	4	1	3	1	8		2	9	4	9	43
36	5	5	1	1	5	2	10		2	9	2	8	50
37	1	7			3	5	3		5	9	3	3	39
38	1	5	4	2	1	2	2		1	7		3	28
39	2	4	1	2		2			3	3	1		18
40	1	3	4	1		3			5	1		3	21
41		5	1	2		1	1	1	3	1		2	17
42		2	4	4		3			2	2			17
43		3	2	2		2		1	5	1			16
44	1	1	1	4		6		1	1			2	17
45		2		2		3		1	1			1	10
46		1	1	3		2		3	3	2			15
47				4				2	1	1			8
48			2	5				1	1				9
49			1					1		1			3
50				2					1	4			7
51								1		2			3
53								2					2
Total	61	59	64	35	61	53	62	55	67	79	65	53	714

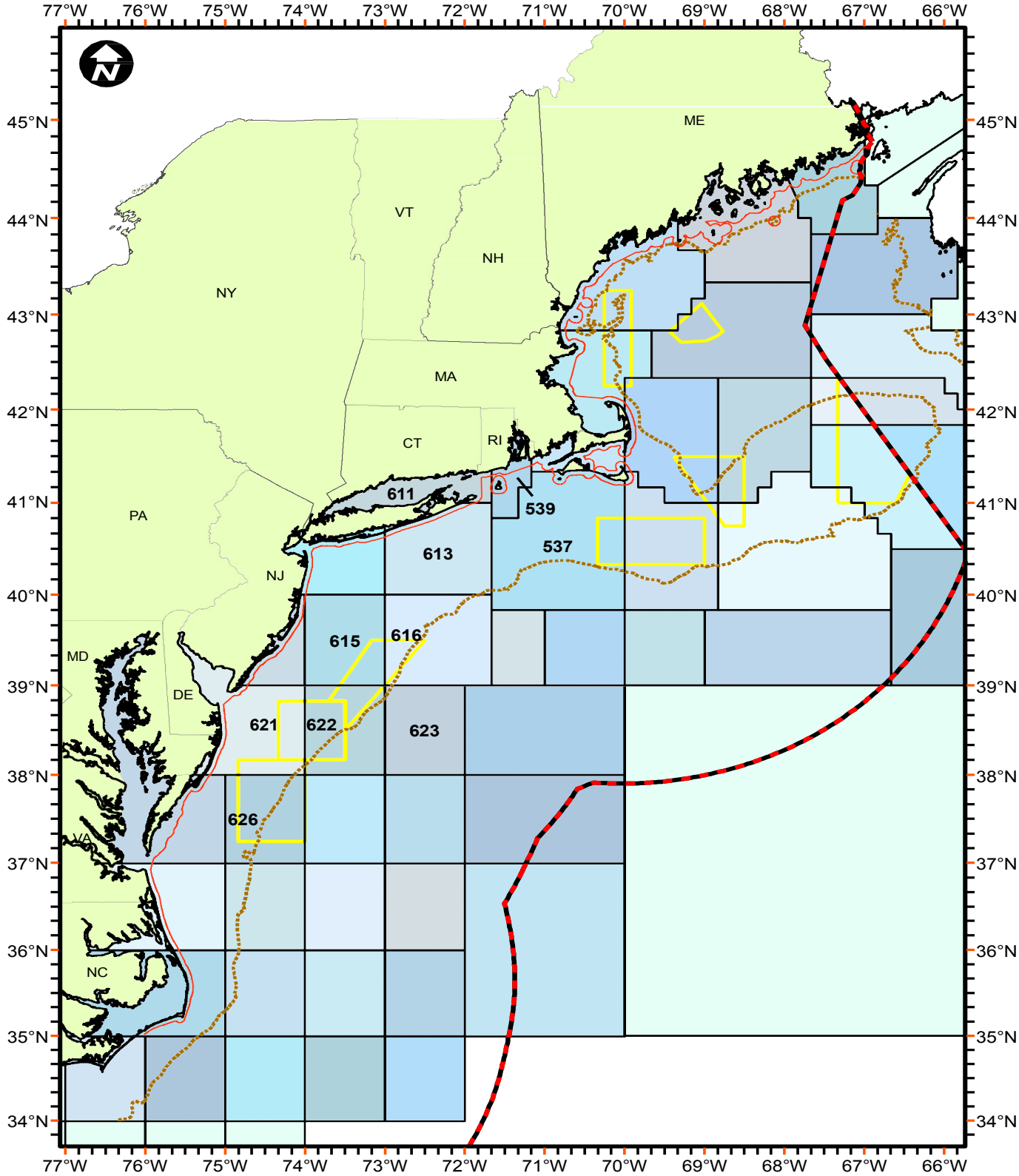

Figure 1. NEFSC statistical areas where black sea bass were collected in 2011 from the US commercial fishery.

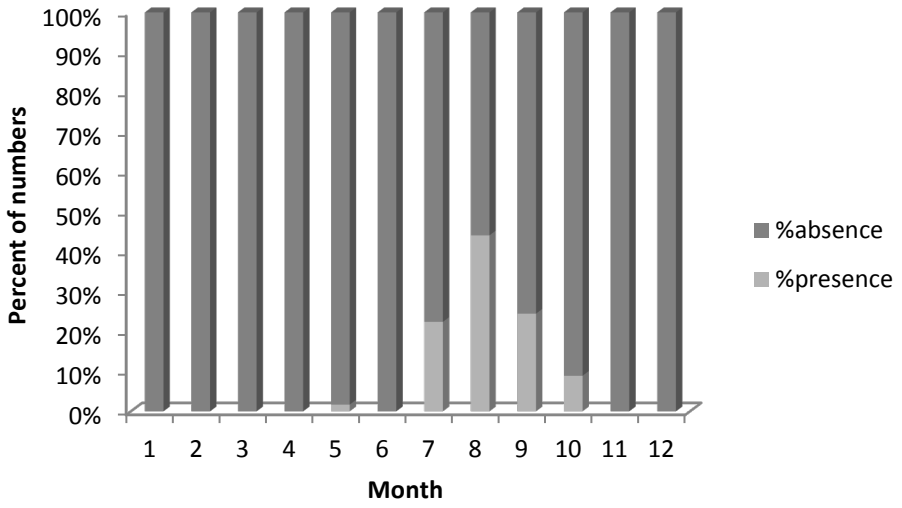

Figure 2. Percent absence and presence of annuli on the margin of scales from age 2 to 5 black sea bass collected in 2011.

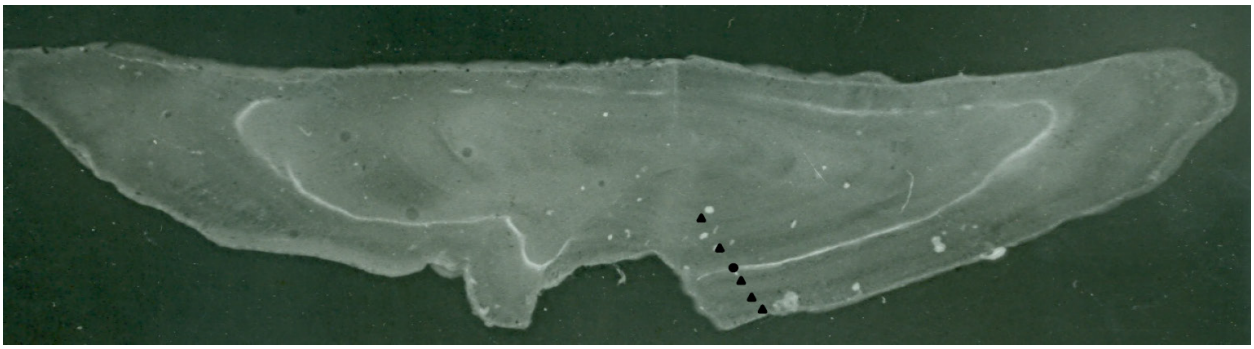

Figure 3. Otolith viewed under ultraviolet light from a 5 year old black sea bass injected with 50 mg/kg oxytetracycline in June 1990 and sacrificed in September 1992. Note distinct fluorescent band resulting from injection (solid circle) and 2 annuli preceding and following the mark (solid triangles).

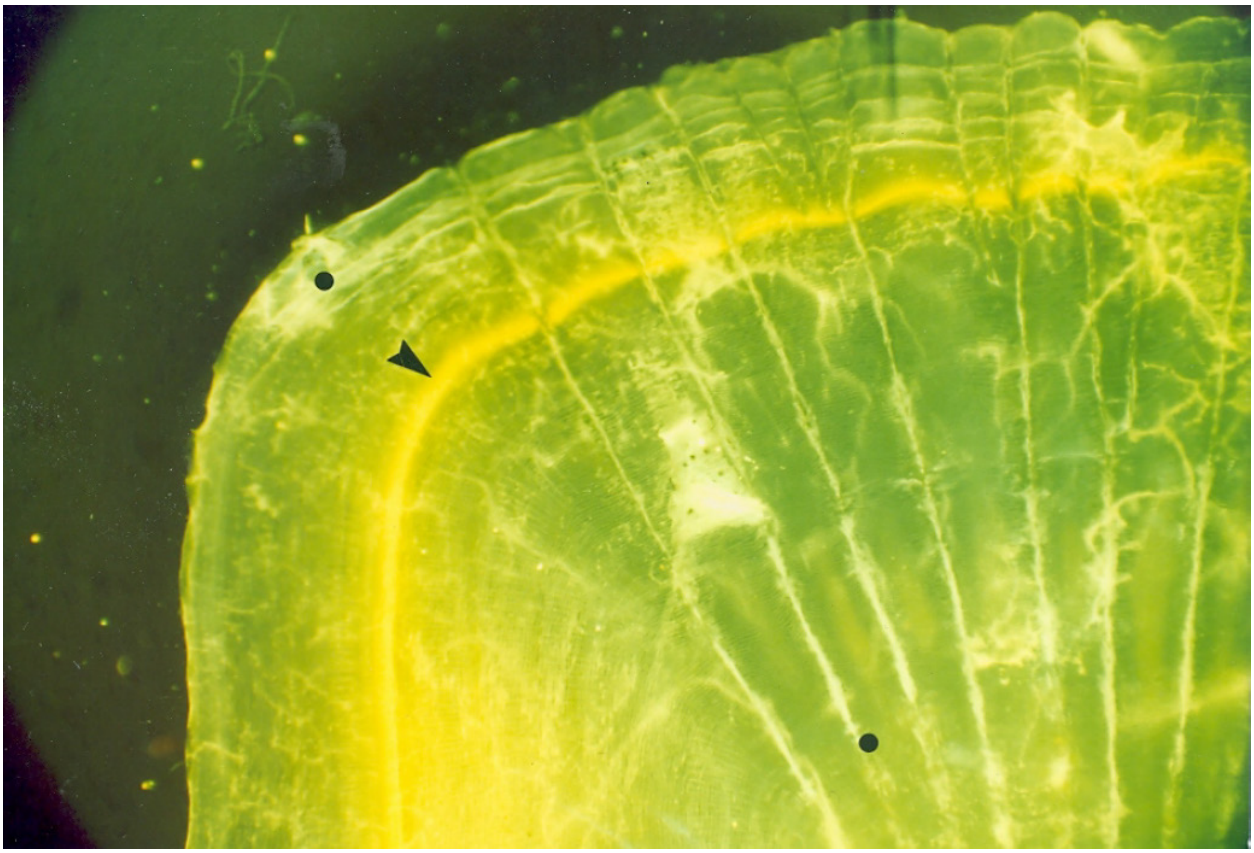

Figure 4. Scale viewed under ultraviolet light from a 3 year old black sea bass injected with 50 mg/kg oxytetracycline in June 1990 and sacrificed in September 1991. Note distinct fluorescent band resulting from injection (triangle) and 1 annuli preceding and following the mark (solid circles).

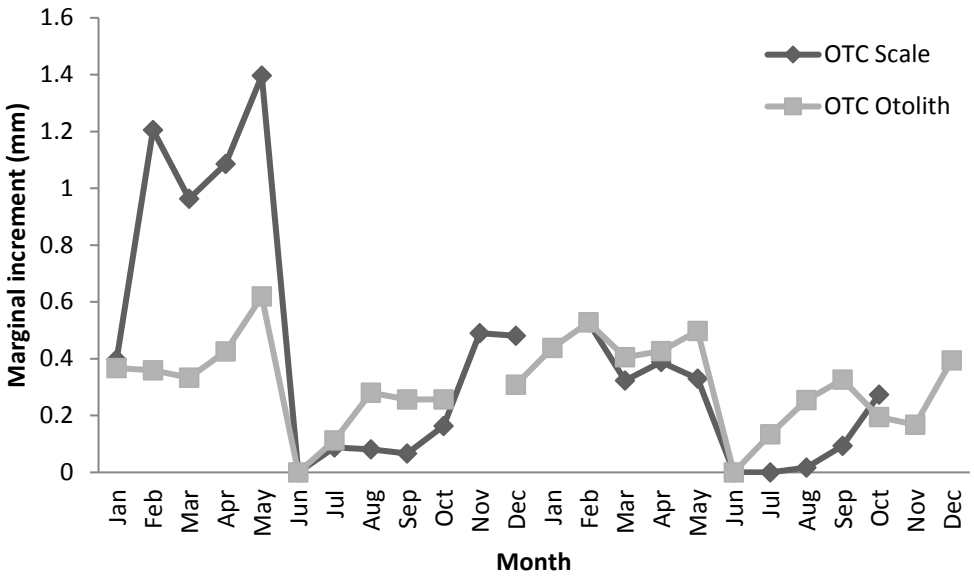

Figure 5. Mean monthly scale and otolith marginal increments measured from laboratory-held individuals injected with 50 mg/kg oxytetracycline in June 1990 and sacrificed at regular intervals during 1991 and 1992.

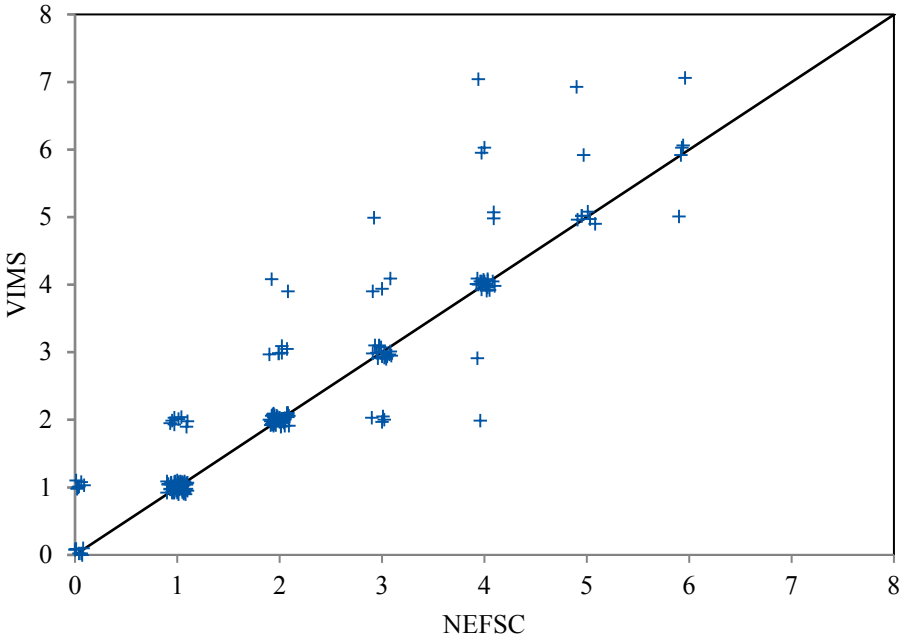

Figure 6. Precision comparison between NEFSC and VIMS original 79.1 % overall agreement. Results showed bias with age 0 samples.

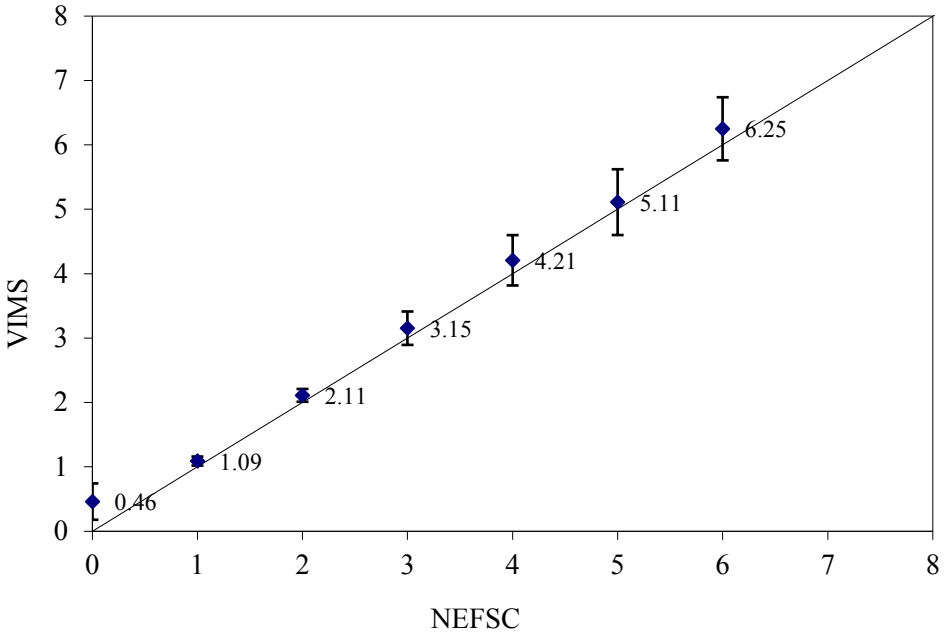

Figure 7. Second precision comparison results after bias was addressed between NEFSC and VIMS. The same samples as above were re-examined, and gave better precision (82.1 %) with less bias. Results still showed bias with age 0 samples.