Report on Age Determination Workshops for Snowy Grouper, *Hyporthodus niveatus*, March 2009 and October 2012

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SEDAR36-WP-09

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Workshop dates: 25-26 March 2009 and 15-18 October 2012

Workshop locations:
<u>25-26 March 2009</u>
Marine Resources Research Institute
South Carolina Department of Natural Resources
217 Fort Johnson Road
Charleston, SC 29412

<u>15-18 October 2012</u>
NOAA SEFSC-Beaufort Laboratory
101 Pivers Island Road
Beaufort, NC 28516

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<u>25-26 March 2009</u>
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<u>15-18 October 2012</u>
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Introduction

In March 2009, state and federal (National Marine Fisheries Service – NMFS) fisheries biologists responsible for supplying age and reproductive data for future assessments of golden tilefish (*Lopholatilus chamaeleonticeps*) and snowy grouper (*Hyporthodus niveatus*) held a workshop at the Marine Resources Research Institute, South Carolina Department of Natural Resources (SCDNR), in Charleston, South Carolina. The biologists at SCDNR are members of the NMFS-funded MArine Resources Monitoring Assessment and Prediction (MARMAP) Program. The goal of this workshop was to standardize techniques of processing life history samples, determining the age of tilefish and snowy grouper, and assessing sex and reproductive state in golden tilefish. This will ensure consistency in age and reproductive analyses among labs in preparation for the SEDAR04 update assessment in the South Atlantic, originally scheduled for mid-2010, and for the SEDAR22 benchmark assessment for golden tilefish in mid-2010 in the Gulf of Mexico. Due to SEDAR rescheduling, the SEDAR04 update was postponed and a benchmark assessment was scheduled for only golden tilefish in 2010.

In fall of 2012, finishing the task of standardizing techniques for determining the age of snowy grouper became a high priority again because a standard assessment is scheduled for the summer of 2013. This task was added to the agenda of a workshop to standardize techniques of determining the age of gray triggerfish (*Balistes capriscus*) and blueline tilefish (*Caulolatilus microps*) that took place on 15-18 October 2012 at the NMFS Beaufort lab.

The Snowy Grouper is a long-lived species, reaching ages of at least 27 yr (± 1-3 yr) based on an age validation study that measured the amount of bomb-derived radiocarbon (^{14}C) in the core of sagittal otoliths (Harris 2005; see also Campana 2001). The increment structure used to determine age of snowy grouper is very complex and age readings/estimates are expected to be highly variable between readers, resulting in a low percent agreement in age when examining sagittal otoliths under a light microscope (see Wyanski et al. 2000). Many, what are believed to be, sub-annual structures/growth increments can be seen in otoliths. In addition, Wyanski et al. (2000) noted crystalline areas that obscured growth increments and rounded opaque deformities in transverse sections that distorted increment spacing.

Results and Discussion

Workshop on 25-26 March 2009

Staff at NMFS Beaufort and SCDNR are processing and examining the otoliths in a similar manner: 1) embedding in epoxy resin and sectioning (thickness of 0.5-0.7 mm), 2) gluing section on microscope slides, and 3) examining the otoliths with both transmitted and reflected light, sometimes tilting the preparation for the best resolution. Examination is done without prior knowledge of size, date of collection, and any previous assessments of age. A box of 100 snowy grouper otolith sections, selected by the staff of NMFS Beaufort for SEDAR 04, was examined by NMFS Beaufort readers (Jennifer Potts, David Berrane) and SCDNR readers (Byron White, Josh Loefer) prior to the workshop. The results for this relatively difficult to age species were encouraging because the difference in age assessment between labs was 0 or 1 yr for 40% of the specimens and ≤ 3 yr for 80% of the specimens. A bias was noted in the readings, though, with NMFS Beaufort consistently counting more increments on each otolith than SCDNR. A subset of the previously exchanged otolith sections was reviewed together, and we discussed our different interpretations. We determined that the majority of the discrepancies were partly due to differing interpretations of the structure constituting the first annulus.

In a previous snowy grouper life history study conducted at SCDNR (Wyanski et al. 2000), the researchers had access to three specimens (37, 156, and 172 mm TL) that were very likely young-of-the-year (YOY). The researchers measured the radius from the core of the section to the margin along the ventral axis. They thought that these YOY fish had not started depositing the first opaque zone (using reflected light). The radial measurements on these specimens was compared to the radial measurement to the first annulus in a subsample of 23 specimens assessed as Age 1; in all 23 specimens, the radial measurement to the first annulus was greater than the radial measurement from core to otolith edge in the three YOY specimens. The data from the 23 Age-1 specimens provided an estimate of the range of radial measurement at which the first annulus could be expected to occur. Following a review of the subset of previously aged otolith sections by workshop participants, it was agreed that the first structure to be included in the increment count should be the first distinct opaque zone within the measurement range determined in the SCDNR study that is visible on both the ventral and dorsal sides and across the sulcal groove. Any faint or irregular opaque zones closer to the core should not be included in the increment count. Participants agreed that growth increments closer to the core than the measurement range for the 1st annulus should be considered sub-annuli. After the workshop, Byron White of SCDNR provided NMFS Beaufort with the range of radial measurement to the first annulus.

Similar to the pattern exhibited by many other species, it was recognized and acknowledged by the group that, with relative consistency, the increment become thinner and more closely spaced as snowy grouper become older. Additionally, the group was in general agreement that when counting increments for the snowy grouper the opaque regions should be read and counted as "fields" at lower magnifications (10x-15x) as opposed to counting all bands visible at higher magnifications. With specimens less than approximately Age 12, it is recognized that there is great variability in the appearance and spacing of growth increments. The group was in agreement that reading the dense opaque marking along either the dorsal or ventral edge of the sulcal groove can be of great assistance when determining an increment count. However, due to the variability in increment structure patterns and frequent occurrence of blurred or otherwise distorted regions of the otolith sections, the entire otolith section should be viewed prior to determining an increment count.

Both labs had difficulty assigning an edge type to the otolith sections beyond the level of opaque versus translucent. It should be noted that the data sets from SEDAR 04 did not include edge type data. We concluded that due to the difficulty in aging this species, the difficulty of assigning edge types, and the lack of edge type data in previous data sets, we will use increment counts as a proxy for age in this species. Nevertheless, it was agreed that the edge type should continue to be assessed as the samples are read due to the potential future use of these data and the relative ease of generating these data now versus in the future.

As a follow up to this workshop, each lab will pull 100 otolith sections from their collections and exchange them between the labs. We will then determine what kind of correction factor or age error matrix is best for the previous data sets.

<u>Workshop on 15-18 October 2012</u>

After a 2-yr period of relative inactivity on snowy grouper due to the shift in SEDAR priorities (noted earlier), work on standardizing the interpretation of growth increments in the sagittal otoliths resumed. Shortly after the March 2009 workshop, personnel from NMFS Beaufort selected 200 otoliths for inclusion in a calibration set and those otoliths were examined by readers from both labs, as described above. However, it was decided at the Oct 2012 workshop that the 200 otoliths had not been randomly selected, as all sizes/ages were not represented; therefore, these otoliths should not be used in the calibration set. In addition, the reader from SCDNR with less experience ageing snowy grouper (Josh Loefer) resigned and was replaced by Paulette Mikell. Shortly before the 2012 workshop, SCDNR personnel randomly selected 89 otoliths for examination at the workshop and for inclusion in the calibration set. Participants at the 2012 workshop examined otolith sections from both labs to review and finalize techniques discussed at the 2009 workshop and decided to create a new calibration set containing randomly selected otoliths from NMFS Beaufort (n = 100) and SCDNR (n = 89).

<u>Inter-laboratory calibration</u>

Four readers, two from each lab, examined the 189 otoliths in the calibration set. Twelve (6.3%) of the 189 otoliths were considered unreadable by one or more readers. Bias plots comparing the age readings of two readers revealed minimal evidence of bias (asymmetry), particularly for Ages < 15-18 (Figures 1, 2, and 3). There were indications of bias at older ages, but sample sizes were small, often one specimen per age group. Bowker's test of symmetry confirmed there was no evidence of asymmetry in these pair-wise comparisons of age readings (Table 1). Average percent error (APE) between reader pairs ranged from 9.02-13.13. A second measure of precision, coefficient of variation (CV), ranged from 12.76-18.56%. The values for both of these measures seem acceptable given that snowy grouper is considered difficult to age. Percent agreement between readers was expectedly low, ranging from 24-36%, but the difference in age assessment was $\leq$ 4 yr for 85-95% of the specimens. There was no evidence that precision varied between labs, as the APE ranged from 10.02-13.13 in pair-wise comparisons (n = 2) of age readings within labs versus 9.02-12.34 (n = 4) in comparisons between labs (Table 1).

The APE between all readers for otoliths examined by all readers was 14.13, with comparable values noted for the NMFS (15.01) and SCDNR (13.16) portions of the calibration set (Table 2). There was a high degree of similarity in the APE values between reader groups (NMFS=11.16 vs. SCDNR=11.32) for the SCDNR portion of the calibration set, with more variability noted in the NMFS portion (NMFS=14.90 vs. SCDNR=8.85).

Conclusion

Campana (2001) stated there is no *a priori* value of precision which can be designated as a target level for ageing studies because the degree of precision is a function of the species, the nature of the ageing structure, and the age reader. Workshop participants anticipated an APE in the 10-15% range because snowy grouper are difficult to age. This is in-line with APEs calculated for several other snapper-grouper complex species that are considered moderate to difficult to age, gray triggerfish for example.

Complete results from an age validation study (Harris 2005) and the inter-laboratory calibration study described within the present report have resolved the issues noted in the report from the benchmark assessment in 2004. Age data from SCDNR were not included in the benchmark assessment due to uncertainty that NMFS and SCDNR have the same protocol for determining the age of snowy grouper and preliminary evidence from a bomb-radiocarbon validation study that the MARMAP (=SCDNR) ages could be too low (SEDAR 4 Stock Assessment Report 1, 2004). The Assessment Workshop concluded that the NMFS age data used in the assessment were preferable for determining parameters of the von Bertalanffy growth curves. Complete results from the age validation study, including an independent re-assessment by two readers of the otolith-derived birthdate (via light microscopy) of the 21 specimens, revealed that those birthdates were generally in line with the age determined by measuring the amount of ^{14}C in the otolith core (Harris 2005). The lack of bias in the age readings and the presence of an acceptable APE support combining the age data for snowy grouper from the two primary sources of data in the U.S. South Atlantic region for use in the SEDAR36 standard assessment.

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Table 1. Indices of precision of age assessments (increment count) by reader pair for snowy grouper sagittal otoliths selected for inclusion in a calibration set by NMFS and SCDNR. APE = average percent error; CV = coefficient of variation; NMFS = National Marine Fisheries Service (Beaufort Lab); p = p value for Bowker's symmetry test; PA = percent agreement, ± 1 yr = percent of specimens for which difference in age assessment between readers was 0 or 1; SCDNR = S. Carolina Dept. of Natural Resources.

				Percent agreement (%)					
Reader Pair	**n**	**APE(%)**	**CV(%)**	**PA**	**±1 yr**	**±3 yr**	**±4 yr**	**±5 yr**	**p**
NMFS1 – SCDNR2	177	9.02	12.76	36.16	67.80	90.40	92.09	93.79	0.5024
NMFS1 – NMFS2	177	13.13	18.56	25.42	56.50	78.53	85.31	93.22	0.2985
NFMS1 – SCDNR1	177	10.32	14.59	32.77	62.71	87.57	93.22	95.48	0.3832
SCDNR2 – SCDNR1	188	10.02	14.17	27.66	63.83	87.77	93.09	94.68	0.4112
NMFS2 – SCDNR2	188	12.34	17.45	24.47	61.70	81.38	85.64	92.55	0.1531
NMFS2 – SCDNR1	189	9.44	13.35	34.39	66.14	87.30	94.71	97.35	0.5339

Table 2. Indices of precision of age assessments (increment count) by reader group for snowy grouper sagittal otoliths selected for inclusion in a calibration set by NMFS and SCDNR. APE = average percent error; CV = coefficient of variation; NMFS = National Marine Fisheries Service (Beaufort Lab); SCDNR = S. Carolina Dept. of Natural Resources.

	All oto.			NMFS oto.			SCDNR oto.		
Group	**n**	**APE(%)**	**CV(%)**	**n**	**APE(%)**	**CV(%)**	**n**	**APE(%)**	**CV(%)**
All readers	177	14.13	18.69	93	15.01	19.97	84	13.16	17.26
NMFS readers	177	13.13	18.56	93	14.90	21.08	84	11.16	15.78
SCDNR readers	177	10.02	14.17	93	8.85	12.52	84	11.32	16.01

Figure 1. Bias plots for counts of annual increments in sagittal otoliths (n = 177) of snowy grouper by readers NMFS1 vs SCDNR2 and NMFS1 vs NMFS2.

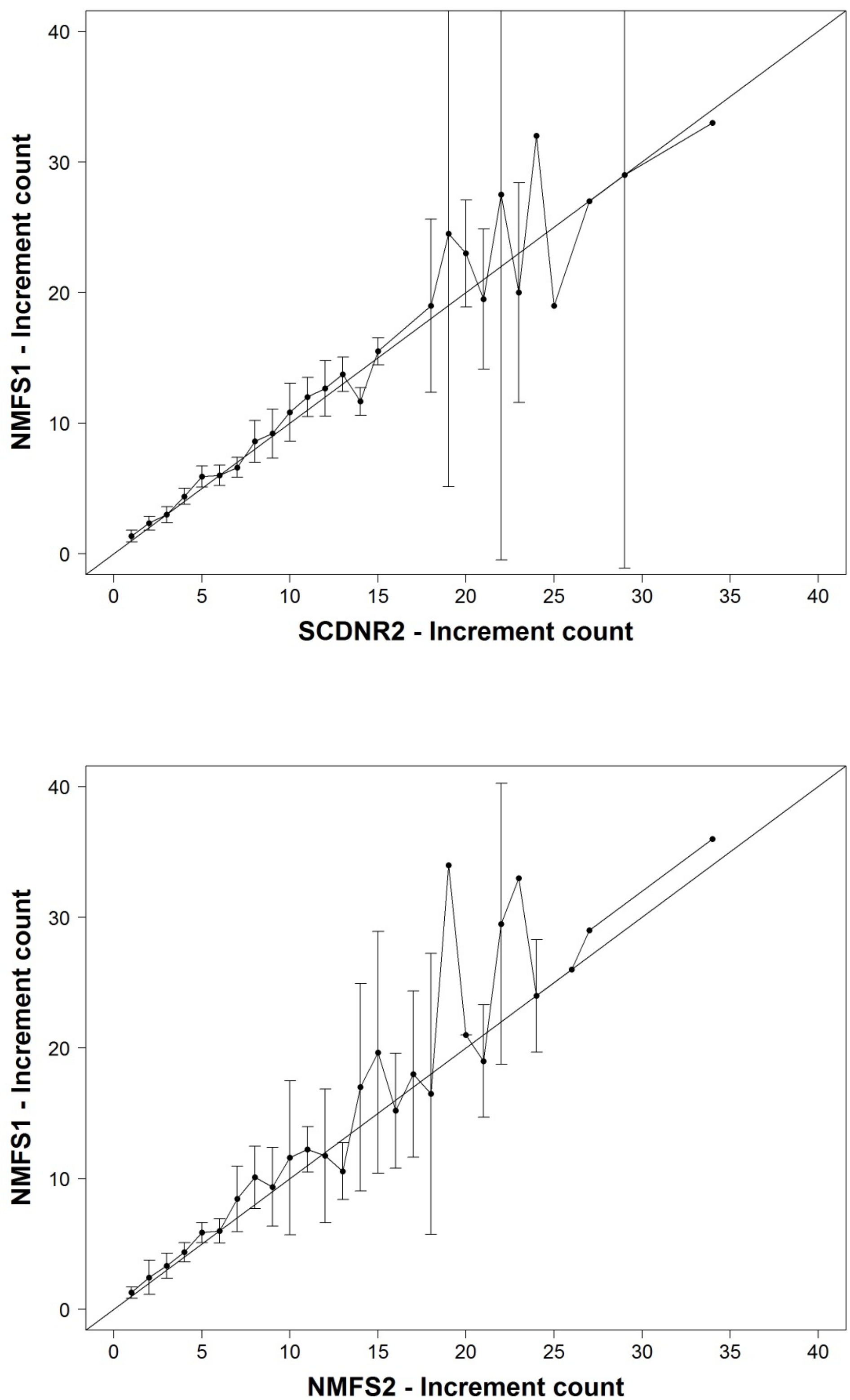
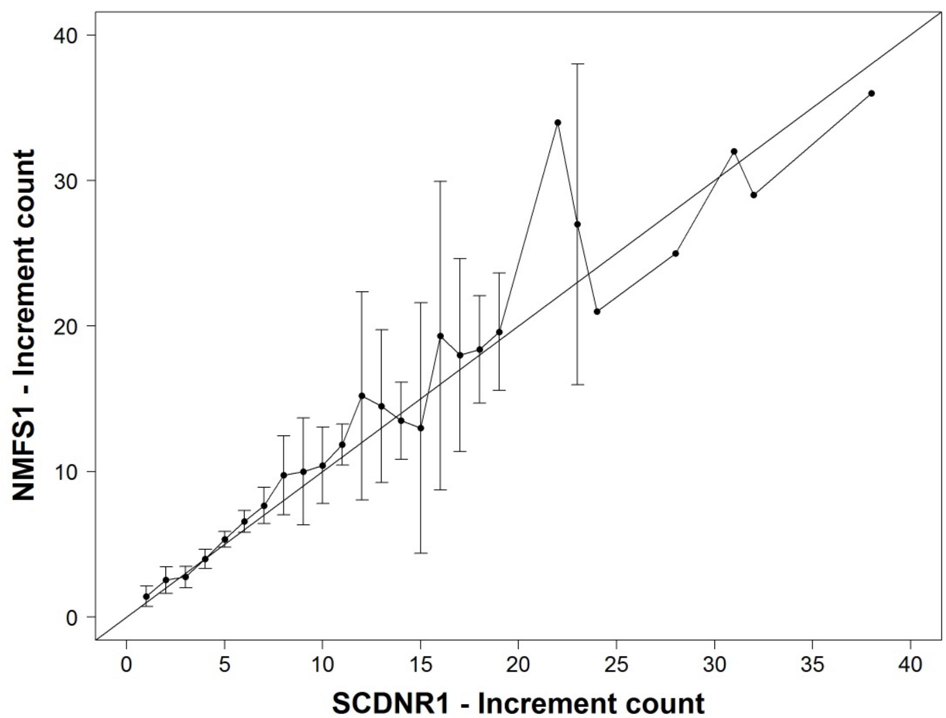


Figure 2. Bias plots for counts of annual increments in sagittal otoliths of snowy grouper by readers NMFS1 vs SCDNR1 (n = 177) and SCDNR2 vs SCDNR1 (n = 188).



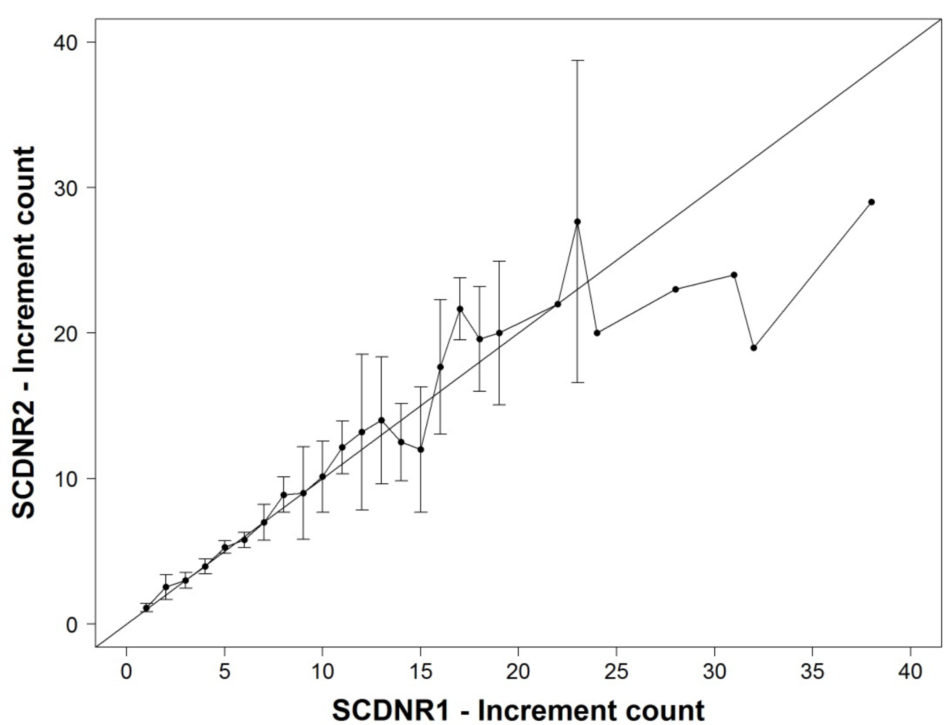


Figure 3. Bias plots for counts of annual increments in sagittal otoliths of snowy grouper by readers NMFS2 vs SCDNR2 (n = 188) and NMFS2 vs SCDNR1 (n = 189).