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Age workshop for red snapper
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Marine Resources Research Institute
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Introduction

The US South Atlantic red snapper (RS) benchmark assessment completed in 2007 utilized data through 2006. Age data for the 2007 benchmark assessment were provided by National Marine Fisheries Service (NMFS) Beaufort Laboratory and South Carolina Department of Natural Resources (SCDNR) Marine Resources Monitoring, Assessment and Prediction (MARMAP) program personnel. The NMFS reader trained with personnel from NMFS Panama City and also compared readings with SCDNR personnel from their reference collection. The resulting APE was 9.65%.

Now with the new benchmark assessment to be done in 2010, these two labs, along with Georgia Department of Natural Resources (GADNR) and Florida Fish and Wildlife Conservation Commission, will be aging RS, and will include data through 2009. Due to the increase in age data providers and change in SCDNR and NMFS personnel responsible for aging RS, we determined that an age workshop was imperative. We must ensure consistency in age analysis between labs in order to merge data sets, thus improving the information for the assessment. Topics covered during this age workshop were methodology for preparing samples for aging, interpretation of the otolith macro-structure, and conversion of increment counts to ages. We will determine the number of otoliths to be exchanged for inter-lab calibration, calculate indices of precision and tests for reader bias. The data will be used to produce an aging error matrix for the assessment model.

Methodology

Consensus among labs for the best processing technique for aging RS is a set of three thin, serial sections around the core, as the sagittal otoliths of RS are relatively large and thick. For expediency, one section encompassing the core can be taken for age analysis. Some quality of age reading may be compromised by taking only one section, but will be minimized by the experience and expertise of the readers. The light source, transmitted or reflected, to be used with the microscope is at the discretion of the reader and should not influence the reading.

In order to assign each fish to the correct year class, the amount of translucent material at the edge, or margin, of the otolith needs to be recorded along with the annuli count. The four labs currently aging RS are using the same edge type codes put forth by SCDNR and used in age studies of other reef fish:

1 Opaque zone on the otolith edge
2 Small translucent zone on otolith edge equivalent to <30% of the previous translucent zone
3 Moderate translucent zone on otolith edge equivalent to 30%-60% of the previous translucent zone

4 Wide translucent zone on otolith edge equivalent to >60% of the previous translucent zone

If any amount of an opaque zone appears on the edge of the otolith, it will be counted. If the fish has not formed its first annulus at the time of capture, an edge type of 4 should be recorded. Because the opaque zones in red snapper otoliths may be broad and diffuse, determining the first annulus and edge type with precision can be difficult. McInerny (2007) noted in her research of red snapper age and growth that the opaque zones were fully formed by the end of May for fish caught off the east coast of Florida, while fish caught off North Carolina and South Carolina finished forming the opaque zone by the end of July. SCDNR and GADNR say the opaque zones were fully formed by the end of July, while FL FWC saw opaque zone completion before the end of June. FL FWC's age samples are collected from fish primarily caught off Florida, thus opaque zone formation before the end of June fits with the conclusion McInerny (2007) made. We need to investigate this phenomenon further and consider adjusting our analysis accordingly.

Another issue with assigning a calendar age to each fish was discussed with regards to which increment counts should be increased by one. FL FWC only increases the increment counts by one if the fish is caught between January 1 and June 30 and has >66% translucent edge, equivalent to edge type 4. SCDNR and NMFS increase the number of increments by one for January 1 to July 31 time period if the translucent edge is >30%, equivalent to edge type 3 and 4. A consensus on when to increase the increment count should be reached. Until such time, data should be corrected appropriately. This issue is most critical in the month of July. To record the calendar age of each fish, the group decided the age of a fish caught between January 1 and July 31 and having an edge type of 3 or 4, will be equal to the number of annuli plus one. For all other fish, the calendar age will be equal to the annuli count regardless of month of capture.

SCDNR brought up an issue with some of their existing age data (n ≈ 2,500). Opaque zone counts were recorded for each sample, but no edge types were recorded. However, radial measurements to each annulus and to the otolith edge were measured on ~800 of those samples. The data cannot be converted to calendar age at this time. The group felt that assigning calendar ages is very important for the stock assessment, because of the signal derived from strong year classes in the stock. The group has suggested that SCDNR re-examine the otoliths collected from January through July to determine the edge type, and then adjust the increment counts to ages.

Acknowledging that a fish continues to grow throughout the year, a fish of a certain year class caught in January will presumably be smaller than what it would have been if caught the following December. To determine the theoretical growth of RS based on observed size-at-age data, the calendar age for a fish will be converted to a fractional age based on peak spawning, a theoretical birth date, and the month of capture of that fish. Red snapper spawn from March through September, with peak spawning in July (pers. comm., D. Wyanski, SCDNR, Charleston, SC), thus July 1 was selected as the theoretical birth date. The observed size-at-fractional age will be used in the theoretical growth model.

Inter-Lab Calibration

For inter-lab age comparisons, SCDNR supplied the workshop with a teaching set of red snapper sectioned otoliths (n = 100). The primary RS age readers from each lab examined the samples and recorded annuli count and edge type for each. These readings were accomplished, the counts were converted to calendar age and the data were compared. An initial APE of 11.3% between readers was calculated. Age bias plots were used to look for systematic differences between the ages assigned by individual readers (Campana et al., 1995). Most of the discussion centered on interpretation of the first annulus. Janet Tunnell of FL FWC has extensive experience reading RS and is calibrated with readers from the Gulf of Mexico. The nature of the first annulus is variable due to the protracted spawning season (Figure 1). The first annulus appears as either a large diffuse continuation of the core (most likely spawned in the fall) or completely separated from the core (spawned in early summer) (VanderKooy, 2003). Allman et al. (2005) validated the formation of the first annulus using juvenile red snapper otoliths from the Gulf of Mexico, and has estimated the distance from the core to the distal edge of the first annulus to be around 1mm.

Three other issues with reading red snapper otoliths came up during discussion and examination of the sections as a group. Some of the readers pointed out that there seemed to be a check mark, or false annulus, on some of the otoliths. It appeared as an incomplete opaque zone on the dorsal edge of the sulcal groove between the 1st and 2nd annuli, but it did not seem to go all the way around the otolith section, nor was it clearly visible on the ventral side. The group decided that we should not count that opaque zone as an annulus. Other discussion ensued concerning the notation of the edge type. Because the opaque zones are not always clearly defined, the reader should look at different areas of the otolith section before deciding on the edge type. Some discussion about the quality of the section was noted. If the section was taken far from the core area and the sulcal groove was grossly skewed, the reader may be missing the true 1st or 2nd annulus. For this reason, the sample should not be read.

Concern has been noted from fishermen about the longevity of the species. Red snapper have been validated to live over 50 years in the Gulf of Mexico (Wilson and Nieland, 2001). The oldest fish from the US South Atlantic was 54 years (McInerny 2007). To verify the oldest fish in samples SCDNR holds, nine samples were selected from SCDNR's calibration set that were presumably twenty years or older. The primary readers from all four labs read these samples. Increment counts ranged from 19 to 38, and the APE was 2.6%. The group felt that the opaque zones on the otoliths were annuli based on prior experience and the results of the validation testing.

Follow-up Work

Following this age workshop, FL FWC sent around their calibration set of 100 sectioned red snapper otoliths. The group decided that an APE of ≤5% would be acceptable (Campana, 2001) assuming no bias between the readings. Overall APE for all age readers on the FL FWC reference collection is 6.15%. It would appear that all age readers became more consistent in interpretation of the structure of the otoliths. No one commented on differences in preparation or quality of the otolith sections in the two reference collections. The age range of the reference collection from SCDNR was 1-10 with 64 fish between the ages of 2 and 4. The age range of the reference collection from FL FWC was 2-32 with 35 of the samples ages 4 and 5, and more fish older than 8 years compared to the SCDNR set. Table 1 shows the % agreement, APE and CV of paired readers and illustrates the improved consistency in aging following the workshop.

Indices of precision that include % agreement, APE or CV are not enough when comparing age readings. None of these can detect reader bias. Campana et al. (1995) suggests using paired reader bias plots about the equivalence line (1:1 diagonal) with 95% confidence intervals, which can detect linear and non-linear bias (Figures 2 and 3). Hoenig (1995) suggests using Bowker X^2 test for asymmetry (Table 1).

Analysis of precision and symmetry for the paired readings show good improvement after the discussion at the age workshop (Table 1). Values of APE for paired readers were all within the ≤5% criteria, except for pairs with reader MARMAP2, though the APE's were just slightly above the 5% value. The Bowker test for symmetry showed all pairs having no systematic bias in age readings after the workshop, as opposed to before discussions. The bias plots (Figures 2 and 3) show slightly different results, though. Initial paired readings did not indicate much bias in the paired readings, except for MARMAP2 who tended to over-age the 1, 2, and 3 year old fish compared to the NMFS reader specifically. When aging the fish from the FL FWC reference collection, which had a much broader range of ages than the SCDNR set, no bias within the 95% confidence intervals was evident. Though no bias was evident, there was some indication that MARMAP2 tended to under-age fish over 5 years old compared to all other readers in the FL FWC reference collection.

Because concern has been raised of the potential under-aging of the oldest fish by reader MARMAP2, who is the primary reader for SCDNR red snapper age data, the group conducted more in-depth analysis of the comparative readings. Eighty-three percent of the age data from SCDNR for the 2010 red snapper assessment (SEDAR24) are between the ages of 0 and 10 years. Using only SCDNR reference collection, NMFS, FL FWC and GA DNR age data were averaged and plotted against the age data from MARMAP2 (Figure 4). No bias was detected, and the data was symmetrically spread across the 1:1 diagonal. Because of these results, no ageing error correction is needed for the data going into the SEDAR24 assessment.

In conclusion, the workshop participants recommend re-reading the SCDNR reference collection to determine if improvement in precision was not a function of the reference collection, but an improvement in overall analysis of the otolith structure. Also, the difference in age readings by MARMAP2 needs to be investigated more thoroughly.

Literature Cited

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Table 1. Paired % agreement, indices of precision, coefficient of variation (CV) and average percent error (APE), and test for symmetry of all age readers of the (a) SCDNR (n=95) and (b) FL FWC (n = 100) reference collections of US South Atlantic red snapper.

a.

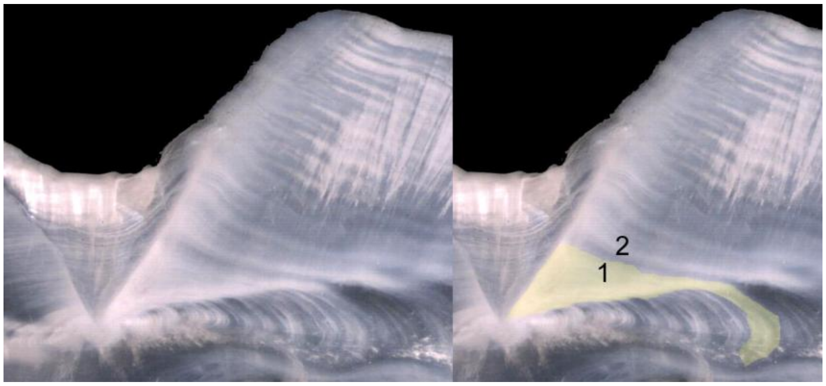
Reader 1	Reader 2	% Agree	CV	APE	Symmetry Test (p-value)
NMFS	Georgia	54.95%	12.28%	8.68%	0.1797
NMFS	Florida	64.84%	10.05%	7.11%	0.0012
NMFS	MARMAP R1	47.31%	14.05%	9.94%	0.5213
NMFS	MARMAP R2	37.36%	19.49%	13.78%	0.0080
Georgia	Florida	85.71%	3.38%	2.39%	0.0430
Georgia	MARMAP R1	78.49%	4.94%	3.49%	0.2383
Georgia	MARMAP R2	46.67%	13.04%	9.22%	0.0115
Florida	MARMAP R1	71.74%	6.03%	4.27%	0.0368
Florida	MARMAP R2	48.89%	12.98%	9.18%	0.2996
MARMAP R1	MARMAP R2	39.56%	16.02%	11.33%	0.0247

b.

Reader 1	Reader 2	% Agree	CV	APE	Symmetry Test (p-value)
NMFS	Georgia	65.66%	4.57%	3.23%	0.1589
NMFS	Florida	83.00%	1.97%	1.40%	0.4433
NMFS	MARMAP R1	64.00%	4.61%	3.26%	0.1754
NMFS	MARMAP R2	47.00%	8.76%	6.19%	0.1175
Georgia	Florida	73.74%	3.44%	2.43%	0.1021
Georgia	MARMAP R1	57.58%	5.82%	4.12%	0.1663
Georgia	MARMAP R2	47.47%	8.39%	5.93%	0.0989
Florida	MARMAP R1	67.00%	4.12%	2.91%	0.2767
Florida	MARMAP R2	49.00%	7.98%	5.64%	0.1268
MARMAP R1	MARMAP R2	42.00%	10.07%	7.12%	0.2348

Figure 1. Thin section of red snapper sagittal otolith depicting the variable look of the first annulus.

a. Diffuse, continuous from the core, first annulus. Most likely fish was spawned in the fall.



b. First annulus separated from the core by a translucent zone. Most likely spawned in the summer.

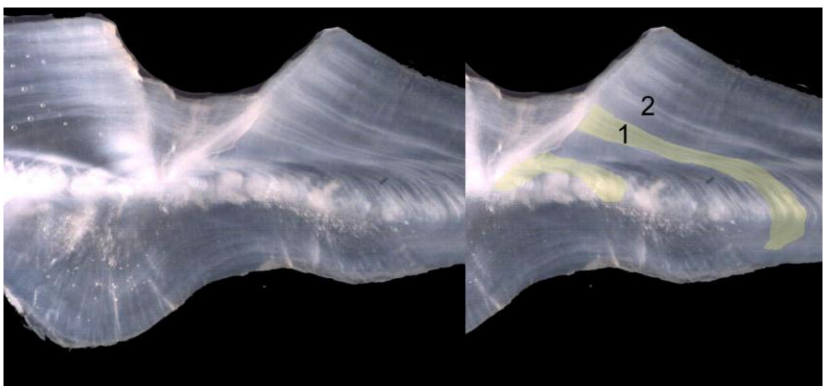


Figure 2. Bias plots with 95% confidence intervals about the 1:1 diagonal of paired age readings of SCDNR red snapper reference collection.

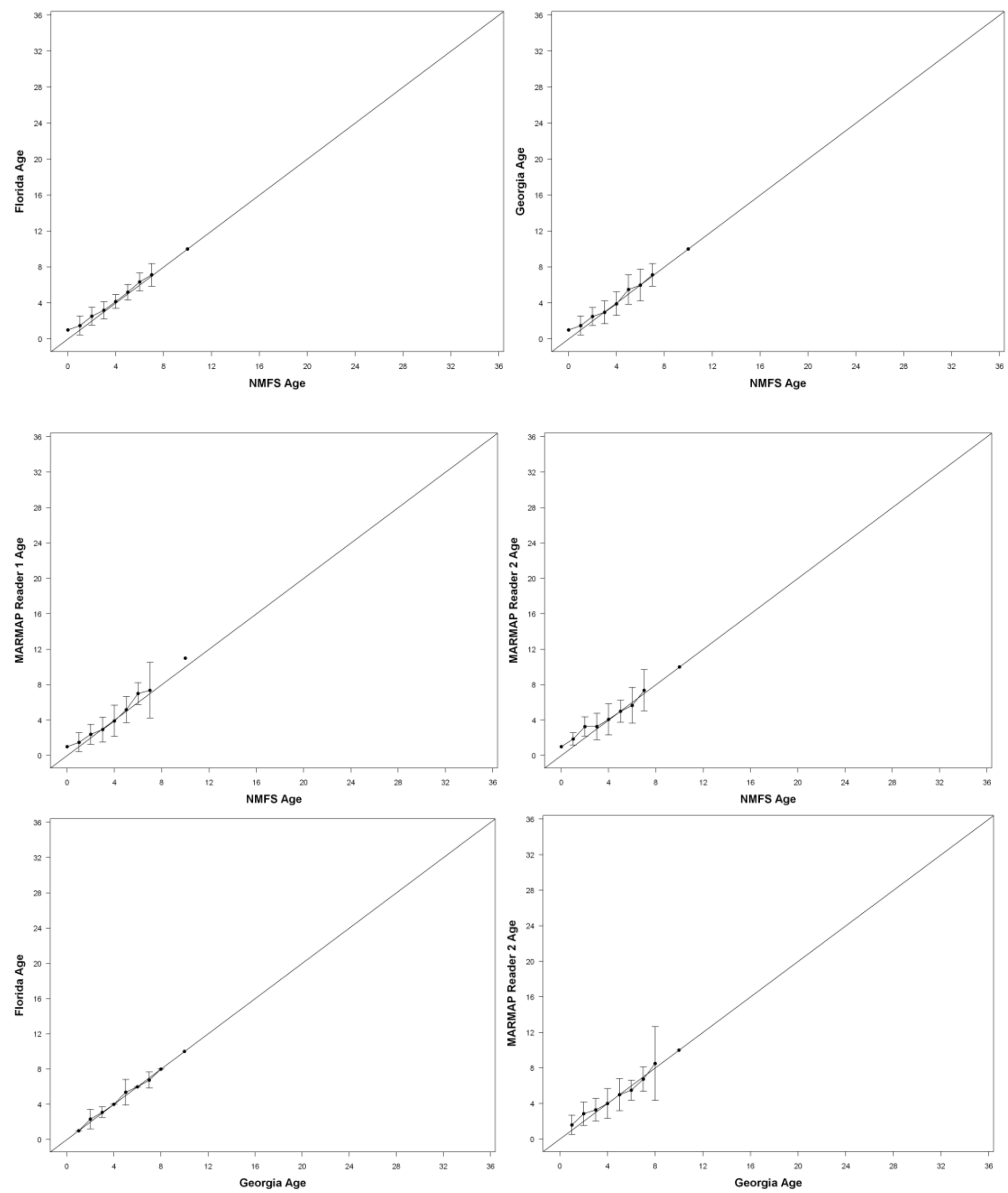


Figure 2. Continued

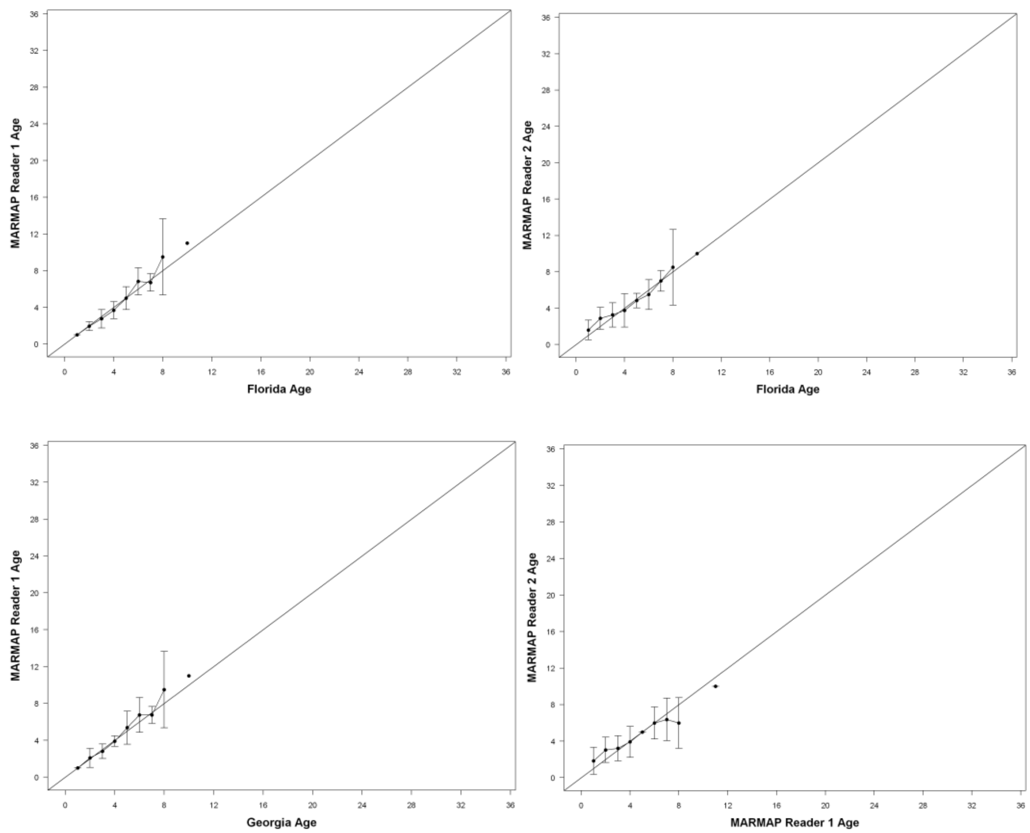


Figure 3. Bias plots with 95% confidence intervals about the 1:1 diagonal of paired age readings of FL FWC red snapper reference collection.

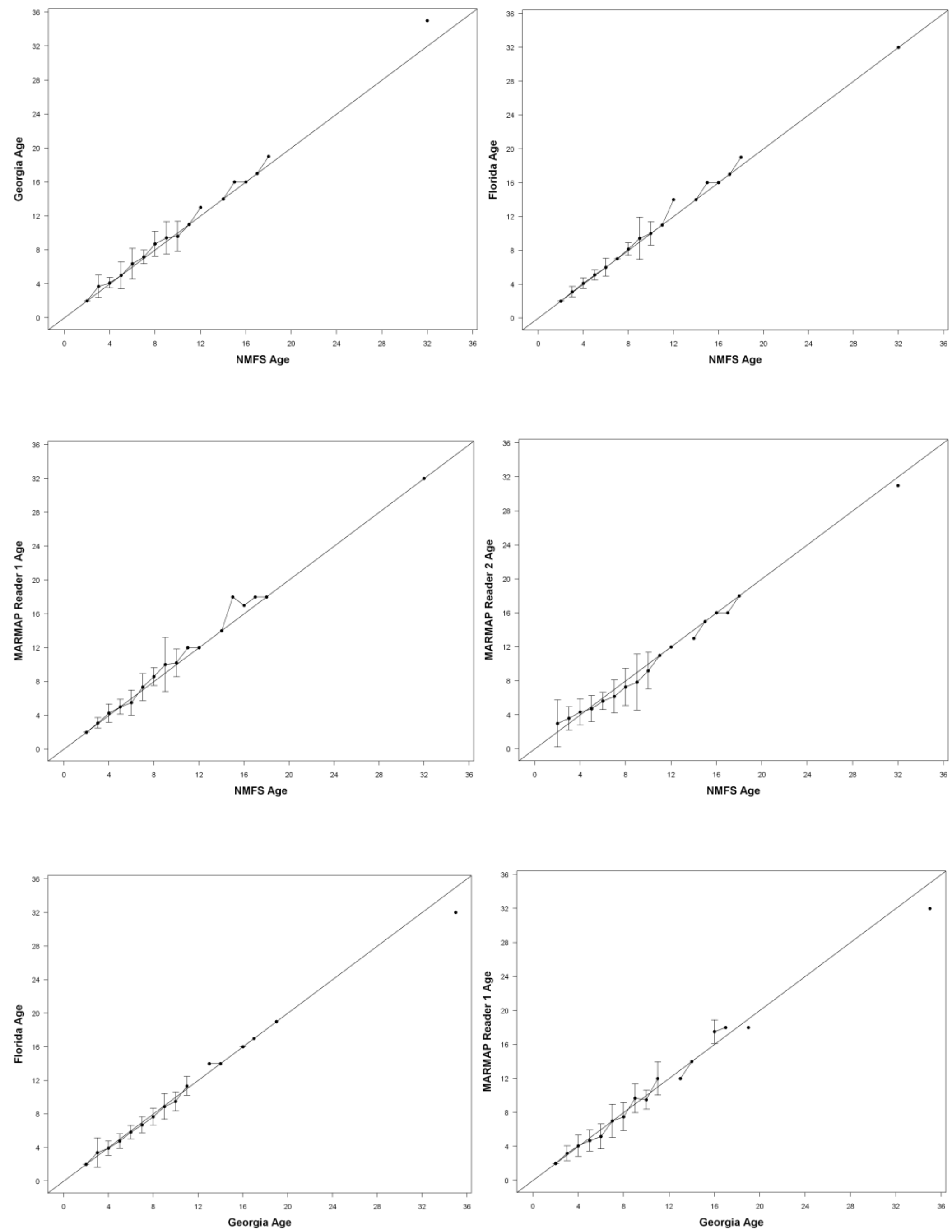


Figure 3. Continued

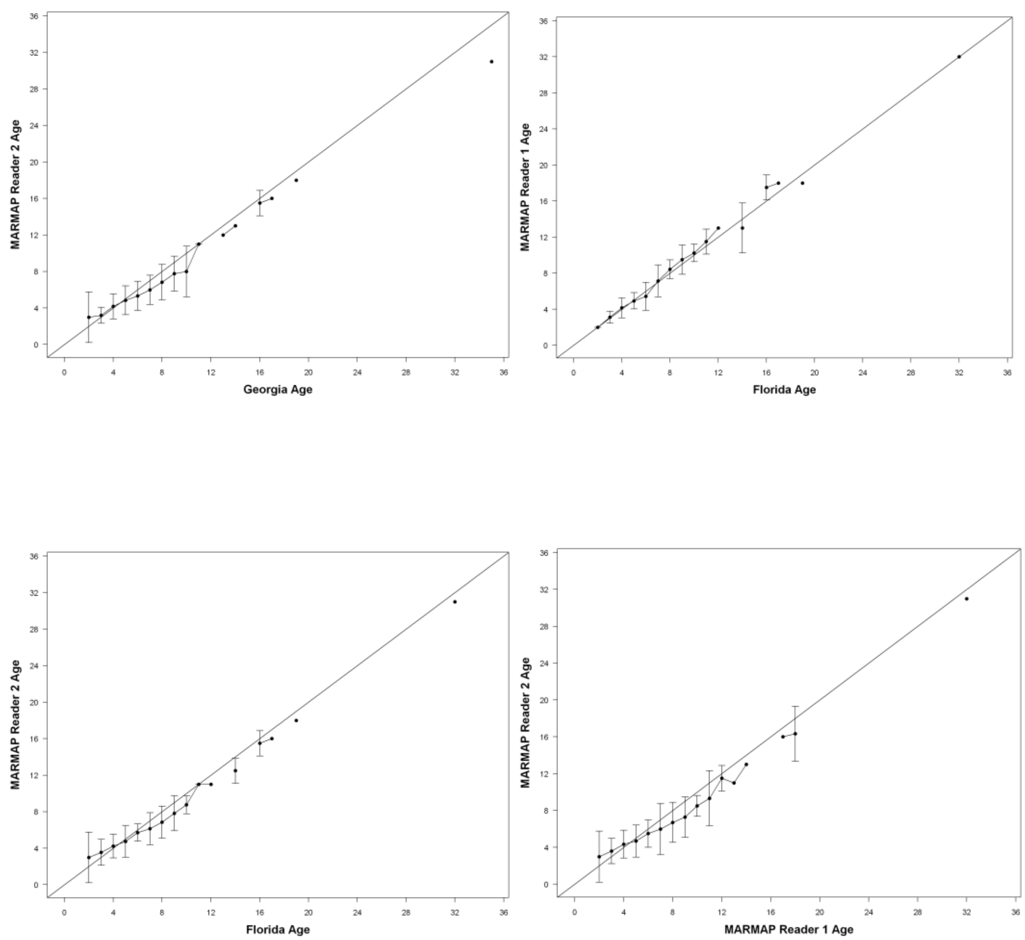


Figure 4. The MARMAP age estimate compared to an average age estimate for the samples from the SC DNR reference collection. The average age is the average from NMFS, FL FWC, and GA DNR, and the line is the 1:1 line.