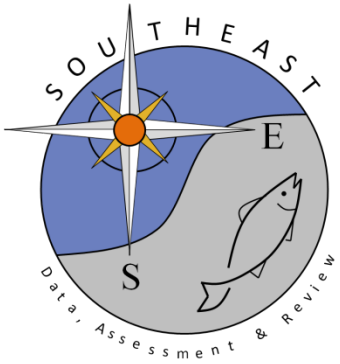
Gray Triggerfish Age Workshop
NOAA's NMFS Beaufort Laboratory, Beaufort, NC
November 19-21, 2013

Jennifer C. Potts,

SEDAR41-RD35

15 July 2014



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By

Jennifer C. Potts

NOAA National Marine Fisheries Service Beaufort Laboratory

101 Pivers Island Road

Beaufort, NC 28557

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List of Participants/Attendees and Affiliations:

Jennifer Potts, NMFS Beaufort Lab, Host*
Lew Coggins, NMFS Beaufort Lab
Michael Cooper, NMFS Beaufort Lab*
Jessica Lewis, NMFS Beaufort Lab
Tracy McCulloch, NMFS Beaufort Lab
Andy Ostrowski, NMFS Beaufort Lab
Erik Williams, NMFS Beaufort Lab

Joseph Ballenger, South Carolina Department of Natural Resources
Tracey Smart, South Carolina Department of Natural Resources*
Adam Lytton, South Carolina Department of Natural Resources*
Amanda Kelly, South Carolina Department of Natural Resources and University of South Carolina Aiken*

Robert Allman, NMFS Panama City Lab*

Virginia Shervette, University of South Carolina Aiken*

* Active age reader for Gray Triggerfish

Terms of Reference

1. Determine best age structure for aging this species – otolith, spine, vertebra.
2. Determine best processing methodology for age structure.
    a. Whole or sectioned.
    b. If sectioned, determine the optimum balance of quality of sections with quantity processed in set amount of time (e. g., multiple sections or one section from each sample, processing equipment, etc.).
    c. Thickness of sections.
    d. Mounting sections on slides.
3. Determine first annulus appearance and range of measured radii from core to first annulus.
4. Define what constitutes an annulus and describe possible check marks or "doubles"
5. Describe edge or margin type of the age structure.
6. Determine timing of annulus formation. Can increment counts be converted to calendar ages?
7. Where do we go from here?
    a. Can a correction factor by applied to one or the other set of samples supplied to SEDAR32?
    b. Which sets of samples would need to be re-read?
    c. Who will do the re-readings?
    d. Of the samples to be re-read, will the labs cross read all samples or portion (e.g., 20%, 50%, etc)?
8. Write up report of workshop.

Introduction

The Gray Triggerfish, *Balistes capriscus*, as a member of the South Atlantic Fishery Management Council's Snapper Grouper Fishery Management Plan, was originally slated for a SEDAR stock assessment starting in February 2013. Prior to the scheduled assessment, the NMFS Beaufort Lab and SCDNR lab started to process and age samples from Gray Triggerfish collected from recreational and commercial fishing trips and gray triggerfish collected by the Southeast Reef Fish Survey (SERFS). NMFS Beaufort and SCDNR housed the collections from the fishery-dependent and fishery-independent sources, respectively. The labs have held two previous age workshops, but still needed to be more

deliberate in coming to consensus on how to interpret the structure of the spines and record the annual increment counts. Details of the first two workshops are in Kolmos et al. (2013).

Gray Triggerfish have traditionally been aged using thin sections from the first dorsal spine. The sagittal otoliths, usually the preferred structure for aging reef fish species, of the Gray Triggerfish are labor intensive to extract and requires cutting through the top of the head to locate, thus diminishing the product for market when sold whole. Also, the sagittae are oddly shaped, and determining the precise plane for sectioning them is difficult. Another age structure of possible use is the vertebrae. If the fish is marketed whole, the vertebrae cannot be readily obtained. In order to acquire adequate numbers of samples from fishery-dependent sources from this species, for practical purposes the dorsal spine will remain the standard age structure used.

Following the second Gray Triggerfish age workshop, an exchange of 200 spine sections was made between NMFS Beaufort Lab and SCDNR. The resulting average percent error (APE) was under 15%, the acceptable criteria established for Gray Triggerfish aging. The test for symmetry also showed no significant bias among readers or between labs (Kolmos et al., 2013). Thus, both labs proceeded with production aging in anticipation of the stock assessment and combined datasets for life history data analyses.

Once the Data Workshop was finished, the lead analyst for the Gray Triggerfish assessment started to plug in the various data to the model. Some of the results of the life history inputs did not make biological sense within the model. The size-at-age and resulting growth rates were markedly different between the fishery-dependent caught fish and the fishery-independent caught fish. Also, the age at full recruitment to the fishery, primarily hook and line gear, and to the fishery-independent survey, primarily trap gear, were also very different. Some of these differences would be expected, but the fishery-independent survey indicated that the fish did not fully recruit to the trap gear until a much older age than those selected by fishery-dependent gear. The assessment model could not fit these data well. Also, the differences in size-at-age between the fishery and fishery-independent survey persisted through the oldest ages where one would expect convergence.

After discussing the discrepancies found in the age data through the initial model outputs, NMFS Beaufort Lab and SCDNR agreed to exchange a new set of 500 sectioned spines for inter-lab comparison. Each lab randomly selected spine sections from fish aged 1 – 8 years. NMFS Beaufort provided 200 samples and SCDNR provided 300 samples. Both labs had their age readers re-age their own samples for comparison, also. Because NMFS Panama City personnel were involved in the first age workshop and have done extensive work on aging Gray Triggerfish from the Gulf of Mexico, the age reader from that lab was sent a subset (n = 300) of the 500 samples to age. The results were mixed. Figures 1 and 2 show the results of the NMFS Beaufort Lab readings of the SCDNR set and the SCDNR readings of the NMFS Beaufort set, respectively. The data clearly show that SCDNR were aging the fish older than NMFS Beaufort. The NMFS Panama City age reader showed a similar trend in that his data showed SCDNR was aging the fish older (Figure 3a). His age readings compared to NMFS Beaufort showed a more mixed

result with his readings being slightly older for the NMFS youngest fish and slightly younger on NMFS oldest fish.

Another interesting result was revealed when each lab re-read its own set of spine sections. The SCDNR re-reading suggested a change in methodology for assigning increment counts. Their new readings were coming more closely in line with NMFS interpretation of the spine structure. NMFS re-readings of its samples suggest that they were slightly more conservative in increment counts of the oldest fish in their original set. Based on all of these results of the exchanges, 30 samples were selected to be imaged and annotated by each age reader prior to the workshop. These images would be a starting point for discussion at the workshop.

The Age Workshop

The workshop opened with a review of the Terms of Reference (TOR). The TORs started out more general to all age workshops and then got into specifics. The "best" structure for aging Gray Triggerfish is the first dorsal spine. This structure was chosen for more practical purposes, rather than necessarily the most reliable structure. Presentations by Robert Allman and Virginia Shervette illustrated their work with triggerfish vertebrae sections. Allman's preliminary analysis of increment counts from vertebrae indicated no bias with fish aged 1 – 4 years from spines, but that the vertebrae ages from fish age-5+ were greater than the corresponding spine ages. His sample size of older fish was small, though, so more work needs to be done. Virginia Shervette's work with comparing vertebrae and otolith sections to the spine sections was also preliminary. Her data also suggest that the oldest fish may be under-aged when using spines versus the vertebrae or otoliths. Because the work from both labs was preliminary, no conclusions could be drawn at the time of this workshop.

Methodology for processing the spines for aging was detailed in great detail in Kolmos et al. (2013). All participants of this workshop noted that more attention needed to be given to the proper thickness of the sections and the location of the sections from the spines. Many spines that were processed by SCDNR prior to the first age workshop were considered to be too thick. NMFS Beaufort Lab agreed to grind the sections thinner on their Hillquist machine to a thickness of 0.5 mm. Several sections were ground thinner during this workshop with good results. It was also noted that taking multiple sections from each spine was preferred to just one section.

The workshop proceeded with a discussion of the 30 images that each age reader annotated to indicate what structure he/she counted as an annular increment. Through the discussion, a set of criteria was developed that each age reader had to agree to use. Everyone remarked on how highly variable the increments on the spines could appear. Some readers may have given the same increment count for a given sample, but were actually counting different features on the spine sections.

Next steps of the workshop were to have each age reader age 14 new samples and compare findings. The sections were imaged and the participants discussed how they interpreted the

increments. Consensus was reached on each sample, the image was annotated with the agreed upon increments, and the process was repeated five more times. Between each set of samples, APE was calculated. The criteria for reading Gray Triggerfish spines were refined during the process and APE values improved and became consistently under 15% between all age readers. Following is a list of the criteria and illustrations of each criteria to be used:

1) Use less than or equal to 20x magnification

2) 1st obvious lobate increment (translucent zone) equal to first increment. The increment must be lobate on both sides of the focus (Figure 6). Use as much of the spine as possible to determine first increment.

3) Valid increments include all obvious bands, where some obvious bands can appear as dark shadows (or negative bands) (Figure 7).

4) When applying criteria regarding discreteness of increments, only look at spine structure posterior to the focus. If two suspected increments merge prior to the focus along the spine margin do not count as separate increments, but rather as doublets (Figure 8).

    a. Structure anterior to the focus should never be used as a primary aging axis

5) Lobes need to be apparent on both sides to include a proposed increment in final increment count. If the increment count on one lobe is less than the other, record the lower number.

6) Generally define edge down the lobes because you have the most resolution.

7) The margin will be noted as translucent (increment) on the edge (code = 1) or as opaque (code = 4). The amount of growth in the spine following formation of the annual increment is highly variable making it difficult to define more specific edge types (e.g., edge codes 1-4 commonly used to define edge type by both NMFS Beaufort and SCDNR in other species).

8) If the spine section appears to be taken in the condyle groove, the increment counts will be most reliably counted out in the lateral plane, rather than down the lobe (Figure 9).

9) Any spine section that has missing parts (e.g., anterior portion, one lobe, etc.) should be excluded from age determination. Many times this issue arises from the extraction of the sample from the fish, rather than sectioning error (Figure 10).

Workshop Follow-up

At the conclusion of the workshop, the two labs agreed to image another 25 spine sections each and exchange the images. Each reader was to assign an increment count and edge code to each sample. The physical samples would not be exchanged due to time delays. A webinar on December 6, 2013 was

held to review the results of the exchange and annotate the images with the agreed upon increment counts. The results were promising with a higher degree of consistency and precision amongst labs. The APE amongst all readers participating in the workshop was 13.9%, with individual reader pair APEs ranging from 6.9-13.1% (avg. = 10.1%). Importantly, all APEs were less than the acceptable APE of 15%.

After adding the last 50 annotated slides to the existing 84 from the workshop, a reference collection of agreed upon ages for 134 Gray Triggerfish had been created. This reference collection will be used by all South Atlantic Gray Triggerfish age readers throughout the production aging process.

To ensure continued consistency in age readings of Gray Triggerfish between NMFS Beaufort and SCDNR, another set of 250 spine sections were pulled by each lab and exchanged. The samples were randomly selected from 1 cm length bins from existing spine sections. Another webinar to review results was scheduled on January 3, 2014. Individual reader APEs ranged from 7.4% to 12.3%, which are all under the 15% criteria set in the first age workshop (Table 1). Bias plots between readers are in Figure 11. There was still some concern over the ages assigned to the oldest fish, though there were very few fish over age 7 in the data set. The group decided that if any one reader assigns an age ≥ 6 to a sample, then another reader has to read it as well. The other discrepancy appears to be centered around age-0 fish. The NMFS Beaufort readers are more likely to give an increment count of 0 to a sample than the SCDNR readers. This discrepancy may only be resolved through an age validation study.

The South Atlantic Gray Triggerfish age readers will start on production aging of the species. Each lab will age 1,000 samples from their own collections and then exchange 500 from each set. If consistency and appropriate level of APE is achieved with no bias, then the labs will continue with the next set of 1,000 samples each. A subset of 300 of those samples from each lab will be exchanged, and again APEs and test for bias will be calculated. Also, for every 1,000 samples aged each reader will randomly select 50 of the reference images, read them, and ensure that he/she is still adhering to the age reading criteria and is consistent with the agreed upon ages. As long as APEs remain ≤15% and no bias is found, then production aging can continue for both labs.

References

Kolmos, K., J. Ballenger, and V. Shervette. 2013. Report on Age Determination and Reproductive Classification Workshops for Gray Triggerfish (*Balistes capriscus*), September 2011 and October 2012. SEDAR32-DW03. SEDAR, North Charleston, SC. 42 pp.

Tables

Table 1. Results of the exchange of 500 Gray Triggerfish spines following the workshop and establishment of a reference set of spine section images.

				% Agreement		
Reader 1	**Reader 2**	**CV**	**APE**	+/-0	**+/-1**	**Symmetry Test**
Jennifer	Mike	10.9%	7.4%	54.7%	88.1%	0.0307
Jennifer	Tracey	14.2%	9.4%	49.9%	83.9%	0.0016
Jennifer	Adam	18.1%	12.2%	40.2%	80.3%	0.0055
Jennifer	Amanda	15.1%	10.4%	46.7%	83.5%	0.0015
Mike	Tracey	15.3%	10.1%	44.7%	81.9%	0.0012
Mike	Adam	18.4%	12.3%	37.6%	78.7%	0.0069
Mike	Amanda	15.9%	10.7%	43.1%	81.5%	0.0002
Tracey	Adam	15.9%	10.5%	44.5%	78.9%	0.7181
Tracey	Amanda	15.3%	10.3%	43.7%	81.5%	0.0000
Adam	Amanda	17.7%	12.0%	40.6%	76.1%	0.0000

Figures

Figure 1. Results of mean age (95% C.I.) of NMFS Beaufort Readers versus SCDNR original SEDAR32 age data assuming SCDNR data represents true age.

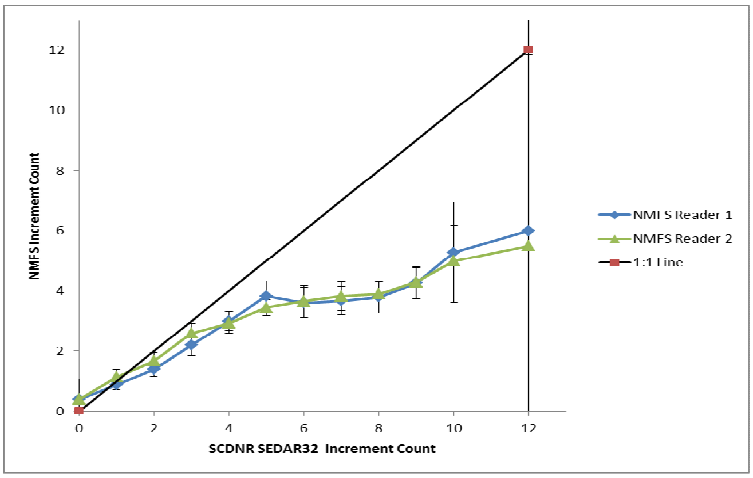


Figure 2. Results of mean age (95% C.I.) of SCDNR Readers versus NMFS Beaufort original SEDAR32 age data assuming NMFS Beaufort data represents true age.

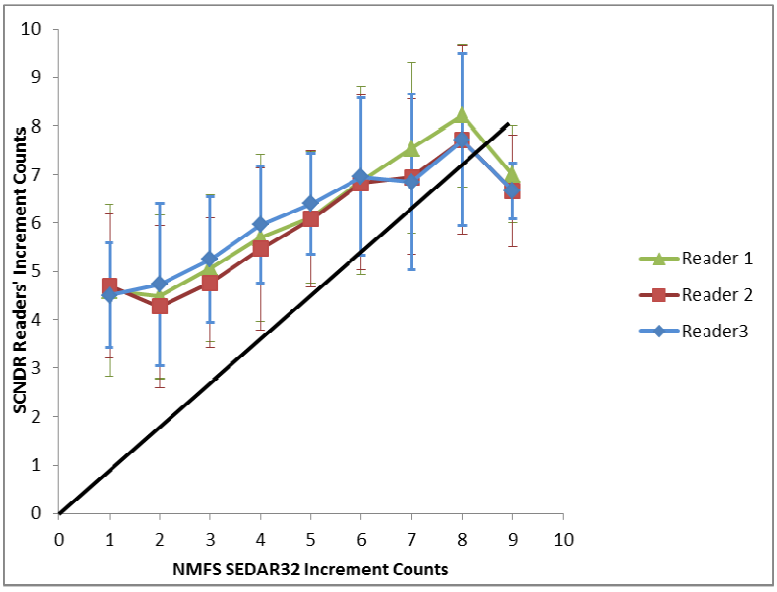
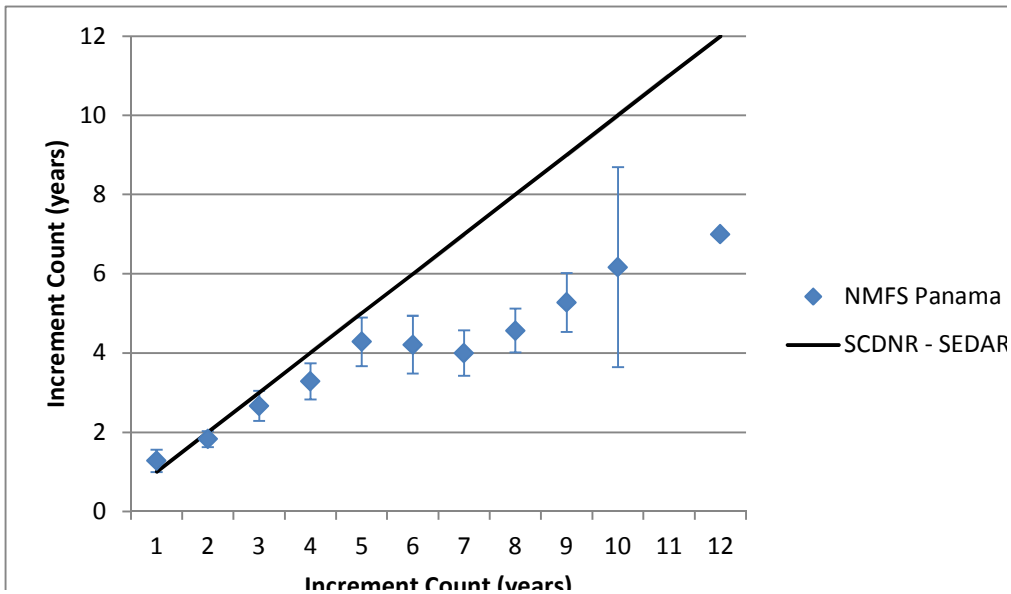


Figure 3. Results of mean age (95% C.I.) of NMFS Panama City Reader versus (a) SCDNR original SEDAR32 age data and (b) NMFS Beaufort original SEDAR32 age data.

a.



b.

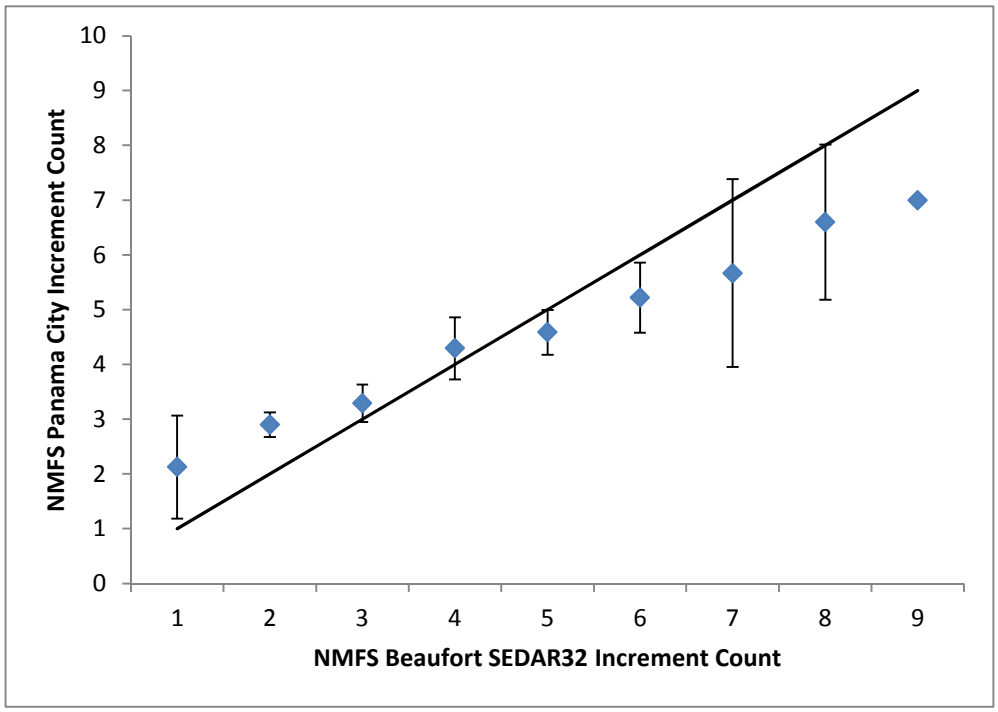


Figure 4. SCDNR re-readings (±95% C.I.) of their original set of spine sections as presented to SEDAR32.

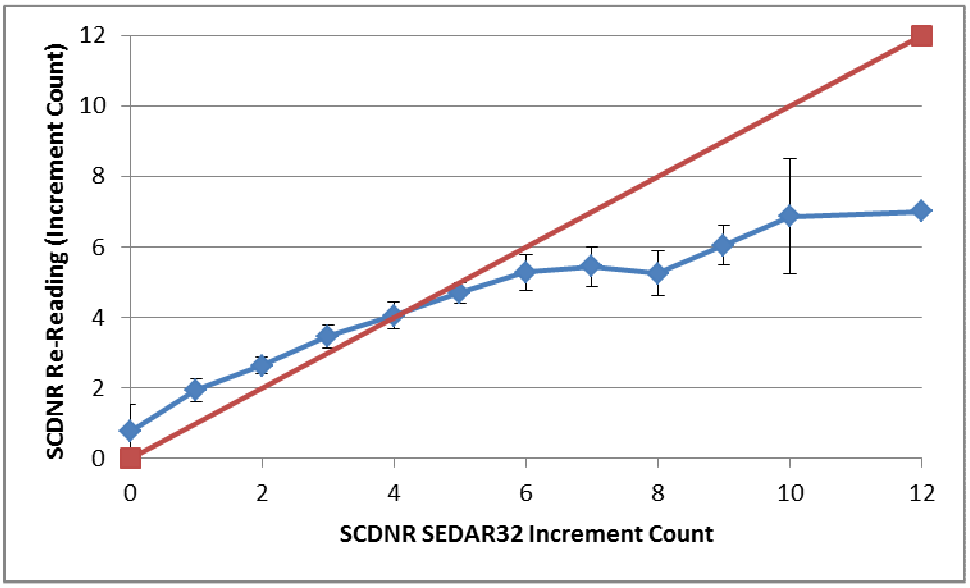


Figure 5. NMFS Beaufort re-readings (±95% C.I.) of their original set of spine sections as presented to SEDAR32.

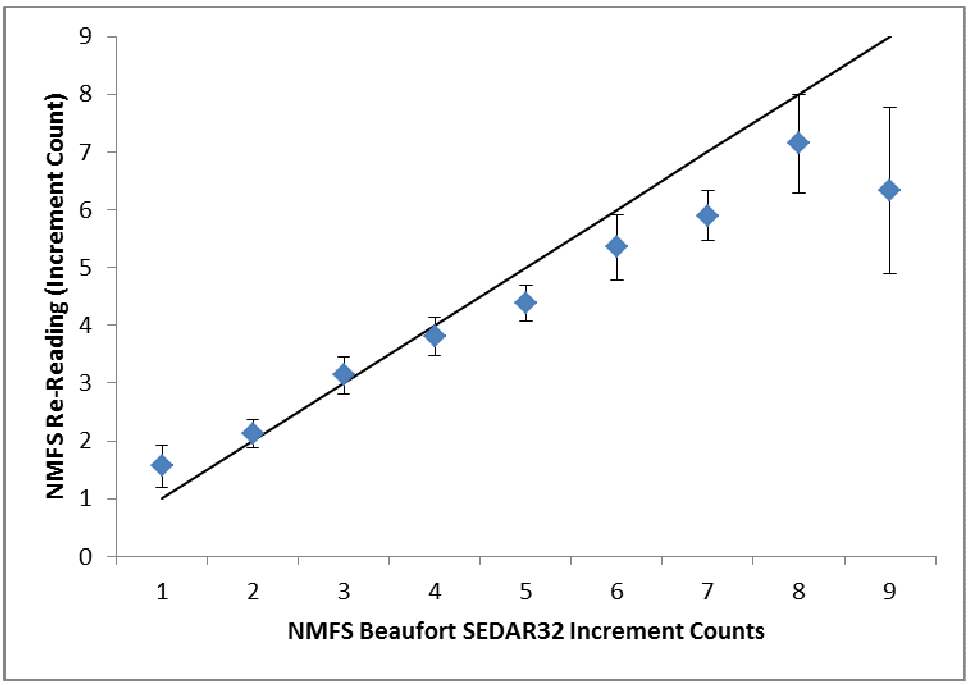


Figure 6. Illustrations of Gray Triggerfish dorsal spine sections and reading criteria for first increment.

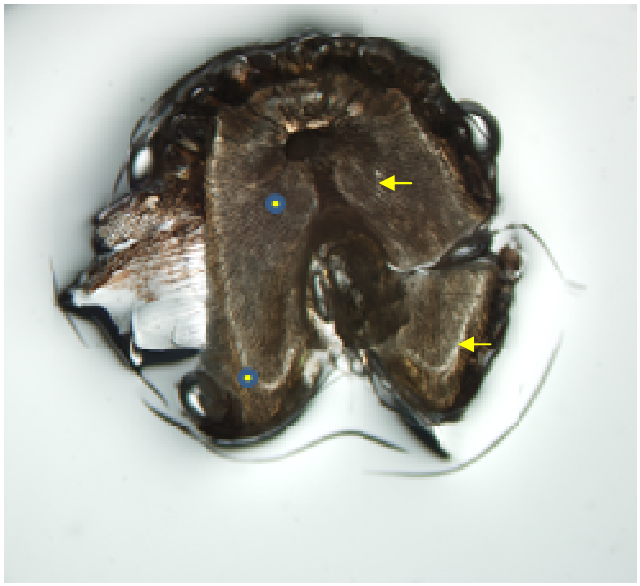


Figure 7. Illustration of Gray Triggerfish increment that can appear dark, or “negative”, rather than bright translucent zone.

Figure 8. Illustration of the area (posterior of red line) of the Gray Triggerfish spine section to use to determine if increments merge as in “doublets” or not. The red dot on the third increment shows the translucent zones merging before the focus. The fourth and fifth increments maintain separation along the lobe.

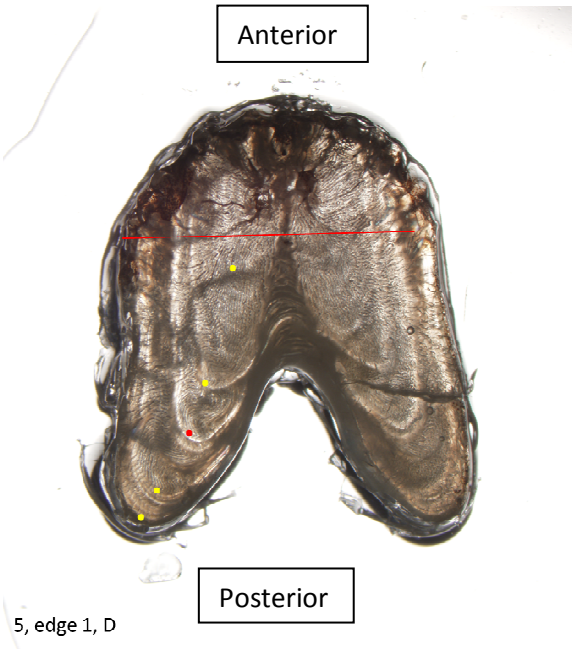
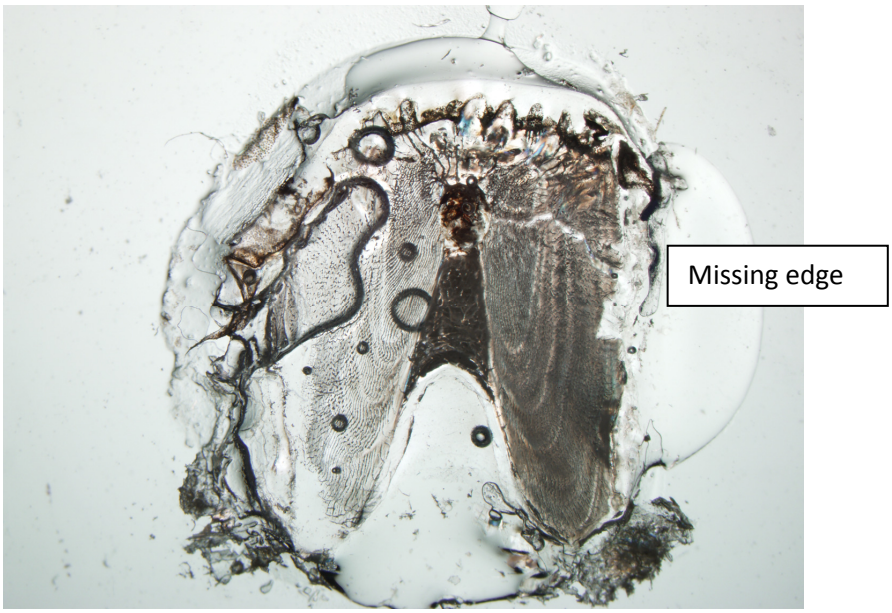


Figure 9. Gray Triggerfish spine section cut from the condyle groove. Increment counts should be observed from the lateral plane, rather than down the lobes.

Figure 10. Gray Triggerfish spine section determined to be unreadable because one lobe is not complete and the other lobe has missing section from the side. The second image illustrates questionable doublets (indicated by arrows) that the age readers could not resolve.



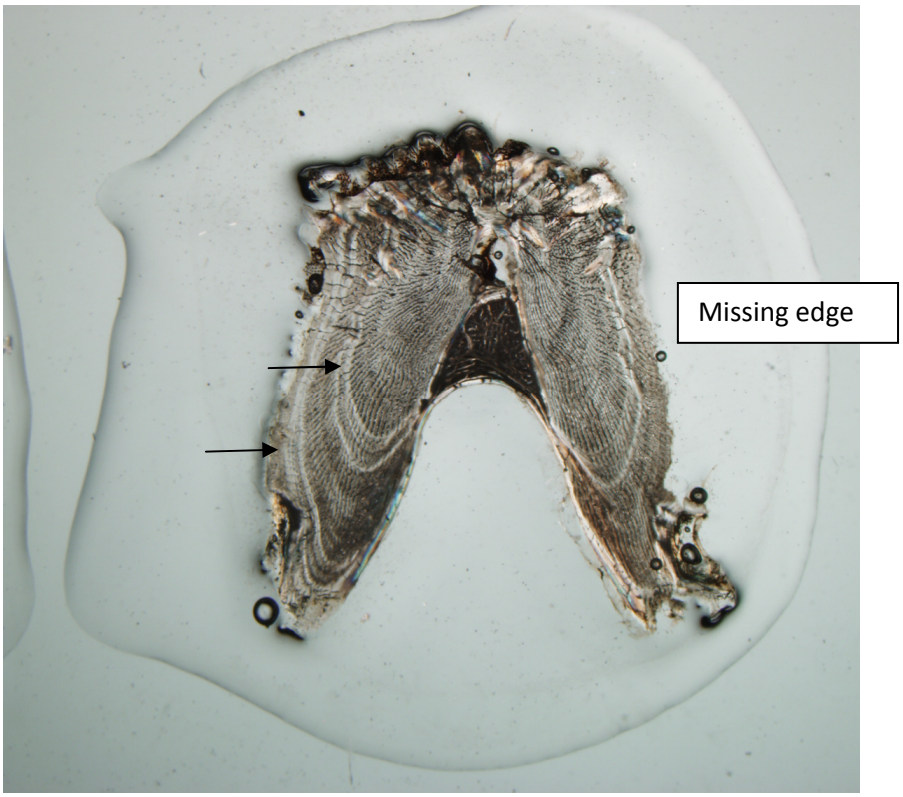
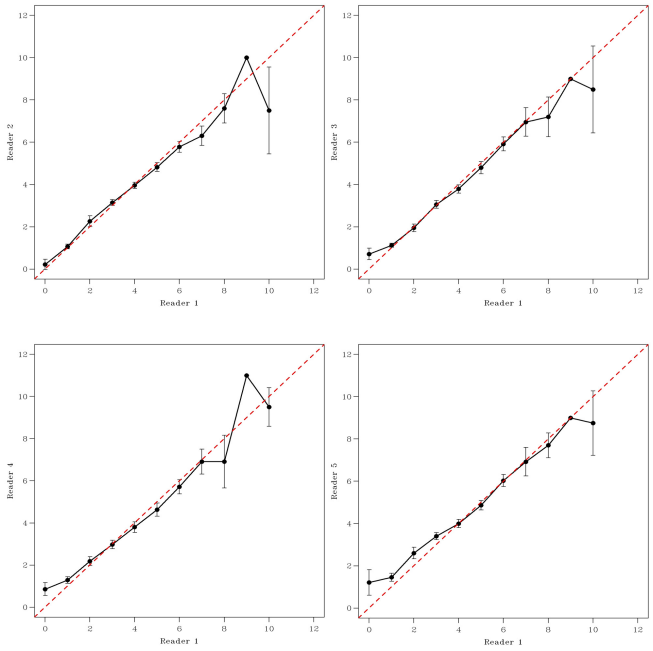
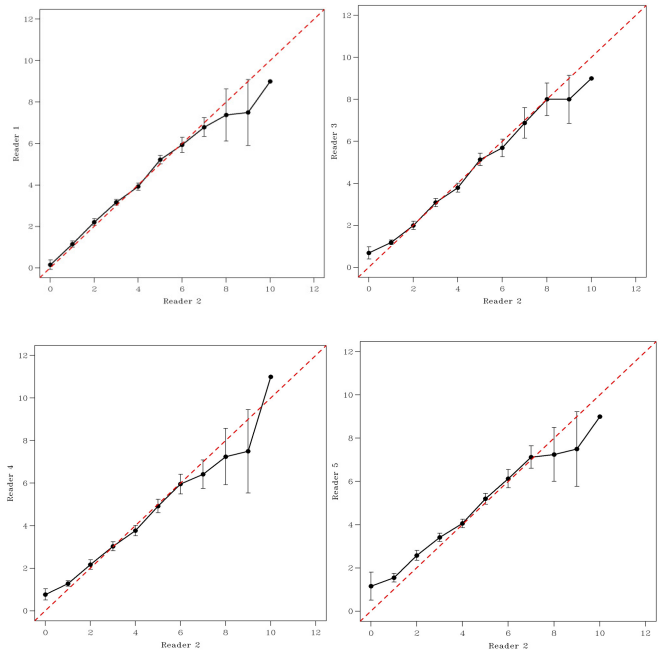


Figure 11. Between reader bias plots of increment counts assigned to Gray Triggerfish spine sections (n=500). Readers 1 and 2 are NMFS. Readers 3, 4, and 5 are SCDNR.

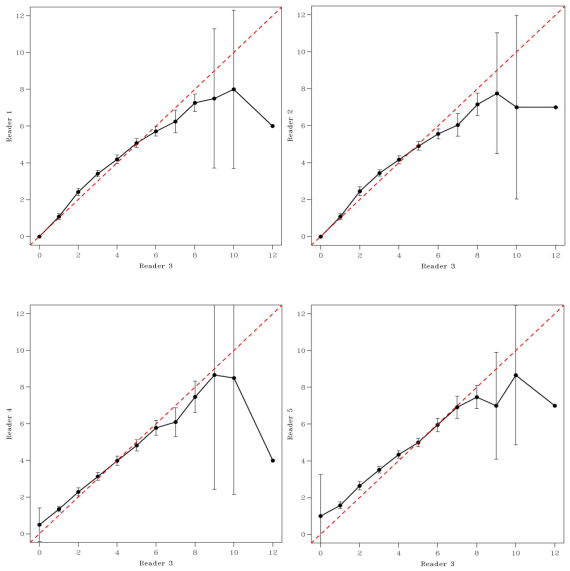
a. NMFS Reader 1 versus other readers



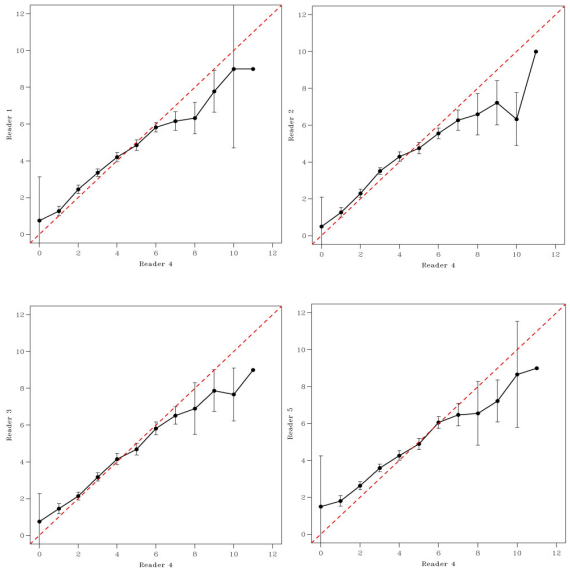
b. NMFS Reader 2 versus other Readers.



c. SCDNR Reader 3 versus other readers.



d. SCDNR Reader 4 versus other readers.



e. SCDNR Reader 5 versus other readers.