Otolith and Histology Interpretation Workshop for Golden Tilefish and Snowy Grouper

March 2009

Agencies:
South Caroline Department of Natural Resources, Marine Resources Research Institute
Charleston, SC

NOAA Fisheries Service, Southeast Fisheries Science Center
Beaufort, NC

NOAA Fisheries Service, Southeast Fisheries Science Center
Panama City, FL

NOAA Fisheries Service, Northeast Fisheries Science Center
Woods Hole, MA

WORKSHOP REPORT

On March 25-26, 2009, state and federal fisheries biologists responsible for supplying age and reproductive data for future assessments of golden tilefish (*Lopholatilus chamaeleonticeps*) and snowy grouper () held a workshop at the South Carolina Department of Natural Resources, Marine Resources Research Institute in Charleston, South Carolina. The goal of this workshop was to validate methods and to compare interpretation of annuli in the otoliths of golden tilefish and snowy grouper, and of reproductive tissues of golden tilefish in preparation for the SEDAR04 update assessment for both species schedule for mid-2010 in the South Atlantic and for the SEDAR22 benchmark assessment for golden tilefish scheduled for mid-2010 in the Gulf of Mexico. Due to SEDAR rescheduling the update SEDAR04 was postponed and rescheduled for a 2010 benchmark assessment in the South Atlantic for golden tilefish.

Participants:
Hope Lyon and Linda Lombardi (NOAA, SEFSC, Panama City, FL),
Tiffany Vidal (NOAA, Woods Hole, MA, M.S. student at Univ. of Massachusetts in Dartmouth)
Jenifer Potts, Daniel Carr, and David Berrane (NOAA, SEFSC, Beaufort, NC)
Marcel Reichert, Paulette Mikell, Byron White, David Wyanski, Kevin Kolmos, Laurie DiJoy, Jessica Stephen, and Jon Geddings (SCDNR, MARMAP)

AGENDA
Wednesday March 25, 2009
9:00am – 11:30am
Introductions, Agenda
Tilefish Aging: Presentation by Linda Lombardi age validation
Discussion about increment interpretation and methodology
Age validation exercise, comparison of age readings
11:30am-1:00pm Lunch
1:00pm-4:30pm
Tilefish Aging (continued)
Concurrent: Tilefish reproduction - reading of histological slides, Dave Wyanski and Hope Lyon
Conclusions and wrap up

Thursday March 26, 2009
9:00am-11:30am
Tilefish Aging and Reproduction (continued) – Presentation by Hope Lyon
Conclusions from discussions on Wednesday and report highlights
Discussion
11:30am-1pm Lunch
1:00pm-3:00pm
Snowy grouper Aging,
Results of earlier readings, examination of otoliths
Discussion

Golden tilefish

Species leaders:
SC: Paulette Mikell, NC: Jennifer Potts, FL: Linda Lombardi

Age and growth
Readers:
SC: Paulette Mikell and Kevin Kolmos
NC: Jennifer Potts and David Berrane
FL: Linda Lombardi

Reproduction
Readers:
SC: Oleg Pashuk and Byron White
NC: none
FL: Hope Lyon

Golden Tilefish Age and growth
- Microstructure used to determine age of golden tilefish is very complex and age readings/estimates are expected to be highly variable between readers. Many, what are believed to be, sub-annual structures/rings can be seen.

- SC, NC, and FL are processing and readings the otoliths in a similar manner: embedding and sectioning, gluing section on microscope slides, examining the otoliths both under transmitted and reflected light, sometimes tilting the preparation for the best resolution. We discussed that often examining the otoliths slightly out of focus may increase the resolution of the annuli. However, Tiffany Vidal (MA) has been reading sections that are not glued to a microscope slide. Byron and Tiffany discussed the SC-DNR protocol and techniques for processing sectioned otoliths.

- Due to the highly variable nature of the otoliths structure it was discussed for the tilefish samples SC abandon the practice of re-examining all otoliths with disagreeing readings for which the two readers disagree on the age. Using the standard SC procedure, otoliths in which no consensus reading can be reached would normally be omitted from the data set. However, due to the complex structure the workshop participants expected that too high a number of otoliths would be removed from the dataset. Rather, the difference between the readers will be used to create a measure of reading variability.
The count/age assigned by the primary reader will be used for SEDAR.

- Although all labs will make notes of edge types (opaque or translucent), there was a consensus that due to the highly variable nature of the otoliths structure, the edge types probably have no consistent meaning. As a result the group recommends that in the assessment, counts are used as a proxy for age (in other words, counts will not be converted to calendar age, or “bumped”). This is consistent with the baseline assessment (CORRECT?).

- Although the variability was high, there was a general consensus on what constitutes an annual increment. These results were strengthened by the validation study. One issue is the potential underestimates for males. This issue was discussed at length and the workshop decided to postpone further discussion until the inter-laboratory calibration readings have been completed.

Golden Tilefish Age Validation

Previous efforts to validate age estimates using bomb radiocarbon dating was inconclusive and led to an application of lead-radium dating, a method that uses radioactive ^{210}Pb:^{226}Ra disequilibria in otoliths as an independent estimate of age (Harris 2005). Golden tilefish were intercepted from commercial bottom longline vessels in 2007 on the east coast of Florida. Both sagittal otoliths and gonad tissue were collected. One otolith from each pair was thin sectioned and the other was cored to the first few increments of growth by grinding the whole otolith. Core samples were pooled based on estimated age and sex (male, female, unknown) into groups that could be analyzed using lead-radium dating. Age was estimated using counts in thin otolith sections and aged independently with lead-radium dating. Radiometric ages closely agreed with age estimates from counts for females and unknown sex fish, which confirmed an annual increment formation (Figure 1, personal communication L. Lombardi). However, radiometric ages did not agree with age estimates from counts for males. This difference may be attributed to differing growth rates by sex. Radiometric results indicated the golden tilefish can live at least 25 years.

Growth increments by sex were measured from core to mid-point of each increment and core to edge of the otolith section along the otolith ventral axis. Mean growth increments by sex revealed similar band deposition until band 7 (Figure 2). There are structural differences in sagittal otolith shape by sex that may explain the divergence of band deposition. Female golden tilefish sectioned sagittal otoliths are stocky with the ventral lobe rounded (Figure 3a). Male sectioned sagittal otoliths are more elongated and the ventral lobe is pointed (Figure 3b) This is only a qualitative interpretation of otolith shape but due to the sexual dimorphic growth of golden tilefish, and the lack of sex data collected from commercial samples, the otolith shape may provided additional classification by sex.

In otoliths from the eastern FL area, the first increment is laid down at about 1 mm from the core (Figure 2). It is expected that a similar pattern will hold for otoliths collected by MARMAP, and MARMAP will complete a limited number of otolith increment measurements to corroborate this pattern. The distance to the core can be used as an aid to determine the location of the first increment (Campana 2001). Structures (rings) significantly closer to the core than 1mm should be considered sub-annuli.

We will circulate 4 boxes (one from each lab, plus one box with otoliths that were used for the validation study) of 100 tilefish otoliths each among the 3 primary reading labs. Four indices were used to determine the level of accuracy between and among readers: average percent error (APE), coefficient of variation (CV), precision (D), and percent agreement (PA) ± 1, 2, or 3 bands (Campana 2001). The results will be used for calibration purposes and to provide data to analyze reading error matrices and biases. The results of this comparison are discussed below.

The workshop recommends that at least a subset of the original preparation (used for SEADR04) is re-examined to investigate potential differences in aging between periods (readers). The MARMAP box for the inter-laboratory calibration will consist of otoliths previously aged by Harris et al.

Participants agreed that for the SEDAR04 update, the last year of age and reproductive data used will be 2010. For each lab, the age and reproduction sections will be read by at least one reader, another reader will read at least a subset, possible the entire data set.

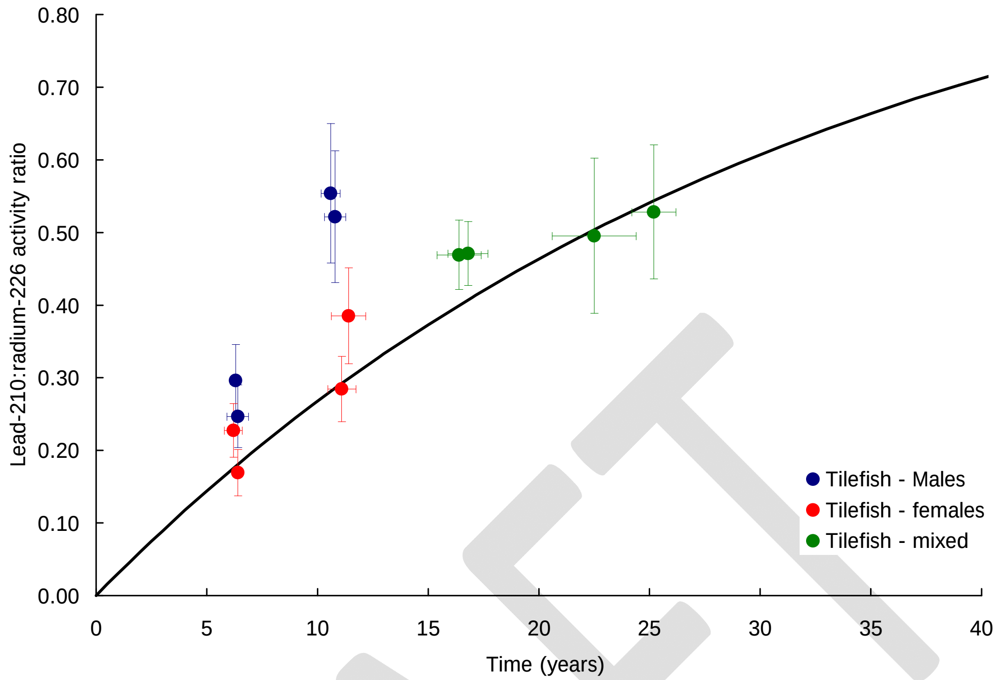


Figure 1. In growth plot for estimated age (mean ± std dev) and lead-radium dating (mean ± std dev) of golden tilefish from the east coast of Florida.

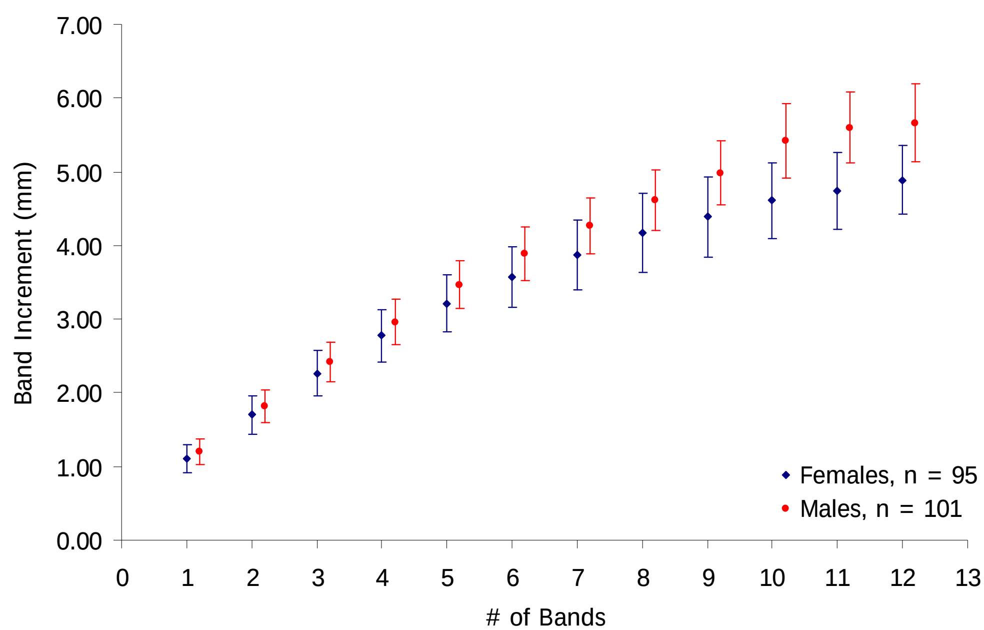


Figure 2. Mean (± std dev) band increment deposition by sex for golden tilefish from east coast of Florida.

Figure 3. Golden tilefish (a) female and (b) male sectioned sagittal otoliths of similar length showing differences in otolith shape (female, fork length = 734 mm, age 26; male, fork length = 758 mm, age 10).

Golden Tilefish Reader Comparison

Five readers (see Table 1) interpreted 288 otoliths (age range: 2 – 35 yrs) with an APE of 11.54%, all readers interpreted 6 otoliths the same (age range: 5-8 yrs), precision (D) of 6.65% and a percent agreement of 70% ± 3 years was estimated among readers (Table 1). The lowest indices of precisions were calculated for the inter-lab (SCDNR MARMAP) readers (APE = 6.00%, PA ± 1 yr = 44%). Reader bias plots provide a vision aid in determine if one reader under- or over- estimates ages (Figure 4a-4j). L. Lombardi and J. Potts had the lease bias reader comparisons (Figure 4j). Low samples sizes of fish age 15 and older resulted in larger variations in reader biases at these ages.

Table 1. Indices of precision for pairs of readers and among all five readers. APE – Average Percent Error, CV – Coefficient of Variation, PA – Percent Agreement ± 1, 2, and 3 yrs.

Reader Pair	APE	CV	PA	± 1 yr	± 2 yr	± 3 yr
P. Mikell - K. Kolmos	6.00	8.48	43.60	75.09	87.54	97.23
P. Mikell - D. Berrane	7.21	10.20	32.87	70.59	84.43	94.81
P. Mikell - J. Potts	9.98	14.12	19.44	60.76	75.69	83.68
P. Mikell - L. Lombardi	8.95	12.65	20.42	60.90	81.66	90.31
K. Kolmos - D. Berrane	8.23	11.64	30.10	64.01	83.04	92.73
K. Kolms - J. Potts	11.09	15.68	15.97	53.82	72.57	81.94
K. Kolmos - L. Lombardi	9.80	13.87	21.11	52.60	77.51	88.58
D. Berrane - J. Potts	9.81	13.88	23.26	56.94	74.65	86.81
D. Berrane - L. Lombardi	9.09	12.85	22.15	61.25	79.58	88.58
L. Lombardi - J. Potts	6.53	9.24	30.21	70.83	91.32	96.18
Overall	11.54	14.87	2.08	20.83	51.74	69.44

Golden Tilefish reproduction

Hope Lyon presented the presence of female reproductive tissues in male gonads and male tissue in female gonads. The pattern was not typical of transitional individuals in hermaphrodites, rather male or female tissues were embedded in the gonads of the opposite sex. The workshop participants agreed with Hope and David, who examined the histological preparations in more detail on Wednesday afternoon that these structures were indeed part of the gonadal structure and not contaminations as a result of sample processing. It was unclear if individuals classified as males or females but also contained tissues of the opposite sex were indeed functional as both sexes. There was some discussion as to the cause of this phenomenon and possible temporal trends. MARMAP has historical samples and once the remaining histological preparations have been examined, potential spatial and temporal trends will be analyzed.

David, Tiffany, and Hope looked at histological slides of Tilefish to determine if there was agreement on reproduction stages from immature to resting, and when the males are spawning capable versus spawning. They were able to determine the areas in the gonads to look for the first signs of development and when the fish begins resting or regressed stage.

Figure 4. Reader bias plots for each pair of readers, mean ± standard deviations with sample sizes above error bars. Red line 1:1 relationship.

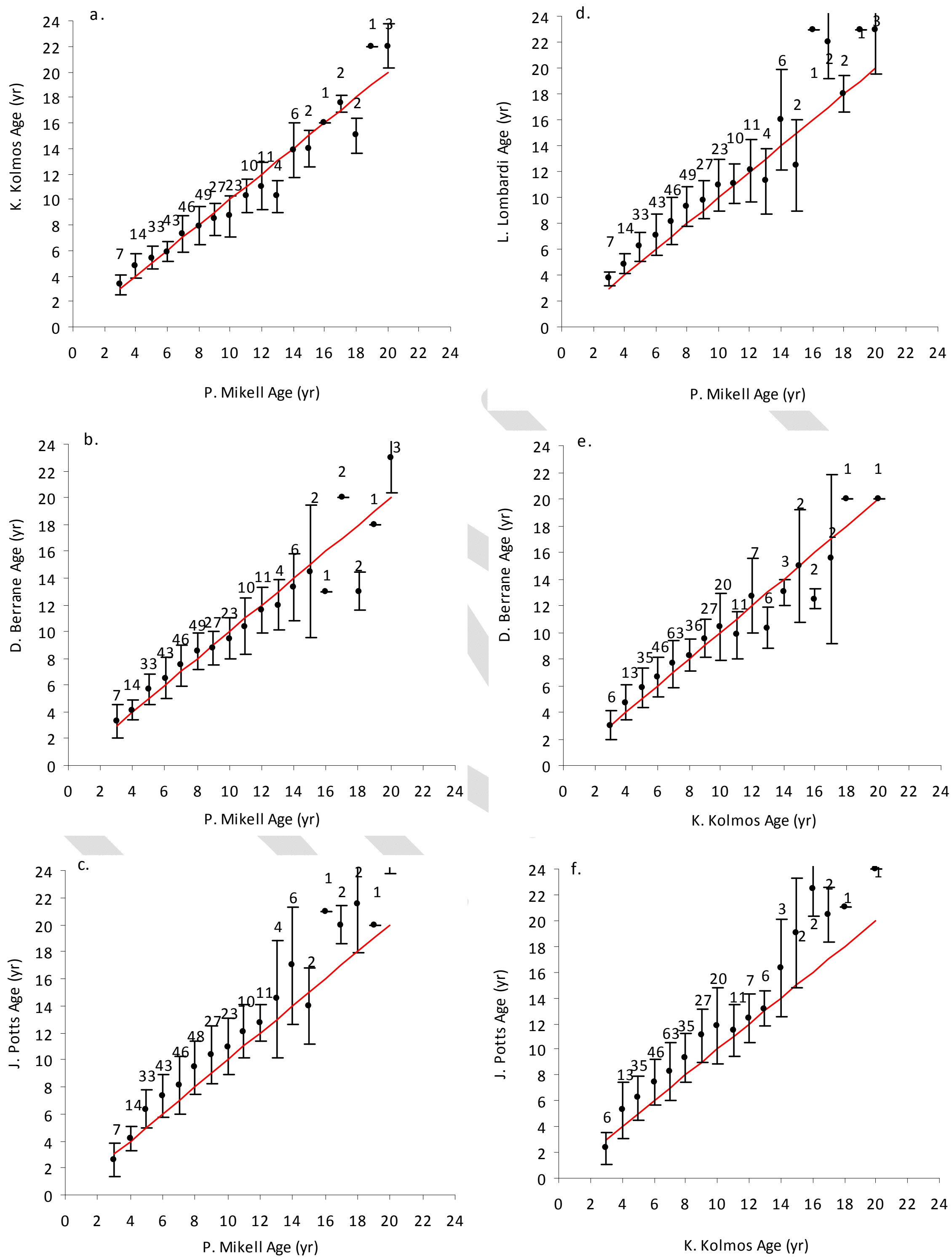
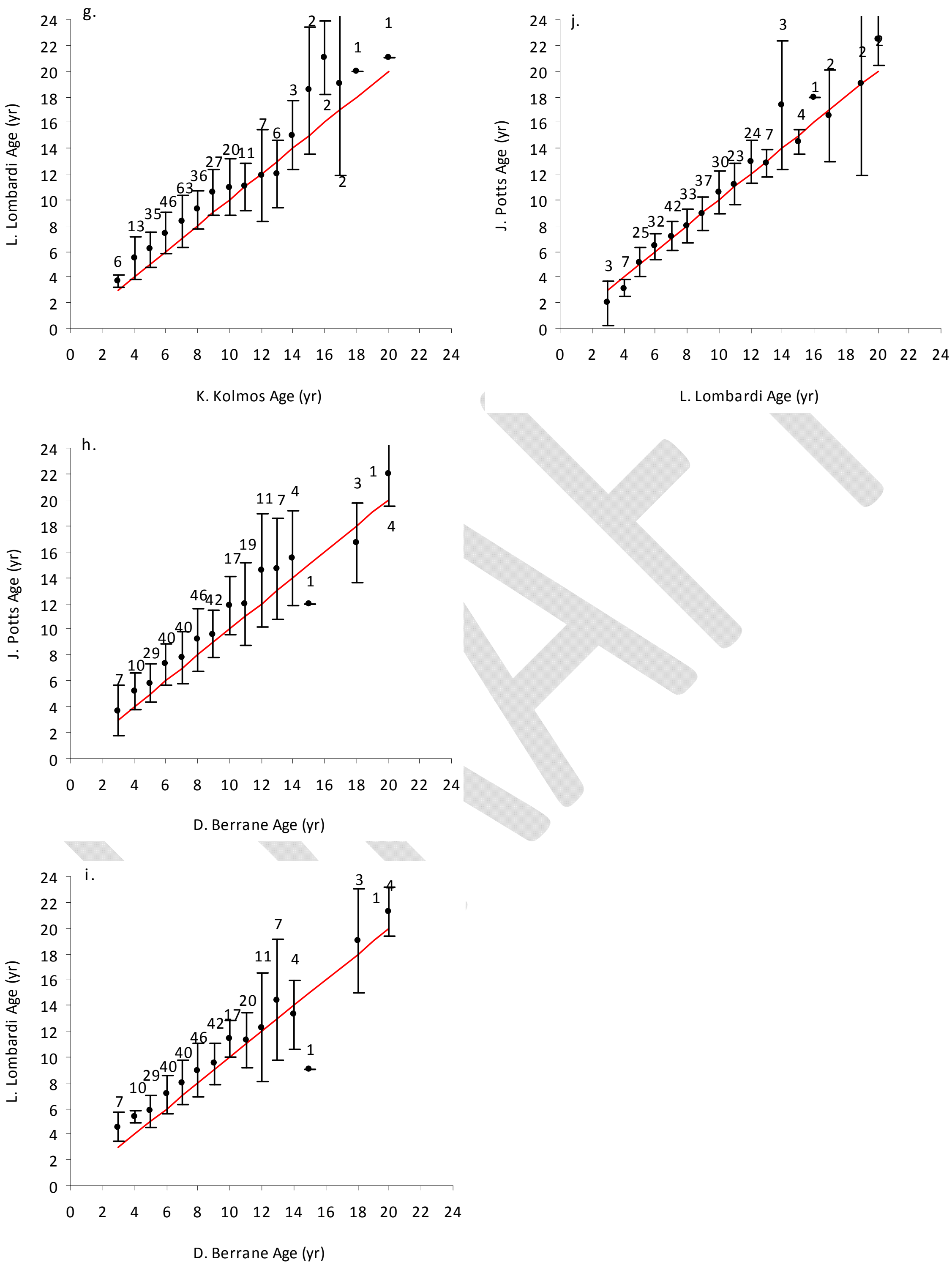


Figure 4 continued. Reader bias plots for each pair of readers, mean ± standard deviations with sample sizes above error bars. Red line 1:1 relationship.



Snowy Grouper Ageing Workshop: 3/26/2009

Species Leaders:
SC: Byron White, NC: David Berrane

Age and growth
Readers:
SC: Byron White, Josh Loefer
NC: David Berrane, Jennifer Potts

A box of 100 sectioned otolith samples prepared by the staff of NMFS Beaufort for SEDAR4 was exchanged between the Beaufort lab and SCDNR prior to this workshop. The results were encouraging in that for this relatively difficult to age species, we had 40% agreement ±1 annulus and 80% agreement within 3 annuli. A bias was noted in the readings, though, with Beaufort consistently counting more annuli on each otolith than SCDNR. A subset of the previously exchanged otolith sections were reviewed together, and we discussed our different interpretations. We determined that the majority of the discrepancies were due to differing interpretations of the structure constituting the first annulus.

In a previous snowy grouper age study conducted at SCDNR, the researchers had access to some specimens that were most likely young-of-the-year (YOY). The researchers measured the radius from the core of the section to the margin in the lateral plane. They felt that these YOY fish had not started depositing the first annulus. The consistency of the radial measurements on these specimens was used to determine a range in which the first annulus could be expected to occur. Following this discussion and the review of the sub-set of previously aged otolith sections, it was agreed that the first structure to be included in the annuli count should be the first distinct opaque zone within the approximate measurement range determined from the SCDNR study that is visible on both the ventral and dorsal sides and across the sulcal groove. Any vague or irregular opaque zones inside this distinct annulus should not be included in the annuli count. Byron White of SCDNR will provide Beaufort with the radial measurements to aid in assigning annuli counts to each fish and be consistent with counts recorded from SCDNR.

Both labs had difficulty assigning edge type of the otolith section beyond opaque or translucent. Also, the existing data sets from SEDAR4 did not include edge types. We concluded that due to the difficulty in aging this species, the issue of assigning edge types and no edge types on previous data sets, we will use annuli counts as a proxy for age of this species. Nevertheless, it was agreed that the edge type should continue to be recorded as the samples are read due to the potential future use of this data and the relative ease compared to generating this data in the future.

Similar to the pattern exhibited by many other species, it was recognized and acknowledged by the group that, with relative consistency, as snowy grouper age the annuli become thinner and more closely spaced. Additionally, the group was in relative agreement that when determining annuli 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 the approximate age of 12, it is recognized that there is great variability in the appearance and spacing of annuli structures. The group was in agreement that reading the dense opaque marking along either the dorsal or ventral sulcal groove edge can be of great assistance when determining an annuli count. However, due to the variability in annuli 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 annuli count.

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.

Citations

Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish Bio. 59:197-242.

Harris, P.J. 2005. Final Report. Validation of ages for species of the deepwater snapper/grouper complex off the southeastern coast of the United States. MARFIN NA17FF2870.