Report of a gag (*Mycteroperca microlepis*) age workshop.

Workshop was held on
September 21-22, 2005
in Charleston, SC.

Participants:
Gary Fitzhugh (NMFS Panama City),
Jennifer Potts, Stephanie McInerny, and Daniel Carr (NMFS Beaufort),
Marcel Reichert, Michelle Bahm, and Mark Collins (MARMAP SC-DNR).

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1 Introduction

On September 21 and 22, a gag aging workshop was held at the SC-DNR in Charleston, SC. Participants were Gary Fitzhugh (NMFS Panama City), Jennifer Potts, Stephanie McInerny, and Daniel Carr (NMFS Beaufort), and Marcel Reichert, Michelle Bahm, and Mark Collins (MARMAP SC-DNR). The goal of the workshop was to compare methods and readings of gag otoliths for age estimates. During the 2 day workshop we discussed reading procedures, data analysis, and otolith structure of gag, in particular the location and structure of the 1st increment and the otolith margin. We also read the otoliths of 503 gag and analyzed the results to compare readings among labs.

2 Discussion conclusions

1) There are only few and minor differences in the interpretation of the otolith structure between labs, irrespective of the type of preparation (whole or sectioned). All participants identified the same structures as the 1st increment and subsequent annuli. Some variability in individual interpretation was present in identifying the edge type, especially in older fish. We concluded that this was predominantly due to a different classification system of the marginal increment among the labs and we felt that this would not affect analysis.

2) All labs are using similar methods during the examination of the otoliths and in the preparation of the slides. Whole gag otoliths are examined under water using a combination of transmitted and reflected light, and regularly the otolith is tilted to aid in the interpretation of the structure. Preparation of sectioned otoliths is done using similar techniques and one or two sections (core area present) of the otolith are examined dry using a combination of translucent and reflected light.

3) All labs are using a system that records the increment count, edge type, and quality of the preparation (see Table 2 for current classification of MARMAP edge and quality type). In spite of slight differences in the classification systems, all were similar in consistently identifying similar edge types for age advancement (e.g. MARMAP's edge type 3 or 4 corresponded with Panama City's "translucent" classification and counts would be advanced if the fish were collected between January1 and June 30).

3) At present all labs examine otoliths whole. Only otoliths of older fish and those difficult to read are sectioned. Depending on the quality of the otolith, MARMAP sections otoliths of fish older than 6-8 years, while the Panama City and Beaufort labs section otoliths of fish older than 8-11 years.

4) All labs are using the same dates and similar edge types for advancing the count of gag annuli to estimate the annual age. For fish collected between January 1 and June 30 with an otolith edge type 3 or 4 (or wide translucent), the increment count is advanced by 1 to obtain the annual age, while fish with an otolith edge type 1 or 2 (or opaque or barely translucent), the increment count is the annual age (no advancement). For fish collected

between July 1 and December 31, the otolith increment count is equal to the age (no advancement).

Table 1
Summary count advancement for gag otoliths.

Collection date	edge type	advance count
Jan.1 – June 30	3-4 or translucent	+1
Jan.1 – June 30	1-2 or opaque	0
July1 – Dec. 31	all	0

5) All labs are using March 1 as the birth date of gag when it is necessary to express annual age in fractions of a year. Fractions (added or subtracted from annual age) would be the difference between the peak birth date and the capture/kill date. Fraction age is commonly needed for growth curve estimations.

6) There are only slight differences in reading procedures between labs.
MARMAP:
At least 2 readers read all otoliths without knowledge of the collection date, length, other sampling information, or results of previous age estimates. Annulus count, the edge type, and otolith quality were noted for the otoliths of each fish (see Table 2 for codes). Results of the readers were compared and otoliths that yielded different readings were examined again by both readers. This second set of readings was then compared with previous ones. If differences persisted the otolith structure was discussed to reach consensus. If consensus could not be reached, the otolith was given a quality code A and the otolith was omitted from the analysis. Since consensus was usually reached, no analysis of reader bias was done. We will continue the process of consensus readings, but are considering including a bias analysis based on the first readings.

NMFS Beaufort:
One person read all gag otolith samples with some knowledge of fish size, but not of the date of collection, location, or gear type. The first 100 randomly selected otoliths were read and assigned a ring count and edge type. After a month had passed, the same person reread the 100 otoliths and data were compared. Since excellent consistency between the two readings existed, the person felt confident to proceed with the rest of the samples. Otoliths that could not be read whole – zones not discernable – were thin sectioned for analysis.

NMFS Panama City:
Otoliths are read without knowledge of the length or weight of the fish. Date of collection is commonly known by the reader. Reader codes are used to define the otoliths edge as having either an opaque margin, a small translucent margin (less than ½ of the previous increment), or having a large translucent margin (greater than ½ of the previous increment). Edge type and date is then used to decide whether the age is to be advanced (from ring count – see earlier discussion). There was a single reader of gag ages at Panama City from 1991-1996 (now retired). Since 1997 there has been a primary reader and for some years, a secondary reader. However there is no protocol for second consensus readings or re-examination of gag ages largely due to the annual volume of otoliths (from several species) prepared and read in the lab. Rather, in-house age

comparison and quality control estimates (precision and bias) have been conducted using a reference collection of gag otoliths. For example, the secondary gag reader only began after meeting a precision target (less than 5% APE, no appreciable bias) in comparison with the primary reader. For the upcoming gag SEDAR (#10), the primary reader (since 1997) has compared ages with the retired reader (for ages used in prior assessments; 1991-1996) and to the secondary reader. These results will be provided in a separate report from the Panama City Lab.

3 Otoliths readings

3.1 Reading/examination of calibration sets

Three sets of otoliths, one from each lab, were examined during the workshop to calibrate readings among laboratories. The Beaufort NC and MARMAP sets comprised of otoliths from 100 fish, and the Panama City set included the otoliths of 203 fish (see Table 3 for data sample summary). Each set was read entirely by Gary Fitzhugh, Jennifer Potts, Michelle Bahm, and Marcel Reichert. A subsample was also read by Mark Collins and Stephanie McInerny, but these readings were not included in the analysis.

3.2 Reading of the whole/section comparison

A fourth set consisted of otoliths from 100 fish (1 whole and 1 sectioned otolith from each fish) from MARMAP samples were read by the workshop participants to compare readings of whole and sectioned otoliths. The 200 preparations were read by Gary Fitzhugh, Jennifer Potts, Stephanie McInerny, Michelle Bahm, and Marcel Reichert and all were analyzed.

3.3 Analysis and results of the otolith readings

Counts were converted to age by advancing or not advancing the increment count where appropriate (see 2.5 and Table 1 for criteria). The average percent error (APE) and coefficient of variation (CV) was calculated (see Stevenson and Campana, 1992) and a series of reader bias plots were made. The APE and CV for the calibration readings was calculated for all readers and in a series of paired comparisons (Table 4). The bias plots represent the paired comparisons only (Figure 1). In the paired comparisons, only those readings were included where both readers assigned a count to the preparation (in other words otoliths that were classified as unreadable by one or both readers were excluded). The overall agreement among all readers was 94.5% APE and 92.8% CV, while the APE was between 95.1% and 97.6% and the CV between 93.1% and 96.7% between any 2 readers. Given the maximum age of the examined fish, we concluded that there was a high level of agreement between all readers.

The whole/section comparison readings were treated similarly, but the APE and CV were calculated for each reader, comparing the readings of whole and sectioned otoliths. The bias plots represent the same comparison (Figure 2). Data were only included if the reader assigned a count to both the whole and sectioned otolith preparation. The APE between the whole otoliths and sectioned otoliths was between 94.9% and 93.4%, while the CV was between 93.7% and 96.3% readers (Table 5). Based on this analysis and

examination of the bias plots, we concluded that there was a high level of agreement between the readings of whole otoliths and sectioned preparations.

Literature references

Stevenson D.K. and S.E. Campana, 1992. Otolith microstructure examination and analysis. Canadian Department of Fisheries and Oceans, 0-660-14747-5, Ottawa, Canada, 126 pp.

Table 2

Readability and edge codes used by MARMAP.

EDGE TYPE

Code	Description	
1	Opaque zone on the edge.	
2	Narrow translucent zone on edge	Width less than about 30% of previous increment
3	Medium translucent zone on edge	Width about 30-60% of previous increment
4	Wide translucent zone on edge	Width more than about 60% of previous increment

READABILITY

Code		Description and analysis consequence
A	Unreadable	Omit otolith from analysis
B	Very difficult to read	Age estimate between readers are expected to be >2 year for young, and > 4 yrs for old fish (>10 yrs) Agreement on age may be difficult to reach, in which case otoliths should be classified as A and omitted from the analysis.
C	Fair readability	Age estimates between readers should be within 2 year in young, and within 4 years in old fish (>10 yrs). Agreement after second reading is expected after some discussion.
D	Good readability	Age estimates between readers should be within 1 year for young, to 2 years in old fish (> 10 years). Agreement after second reading is expected without much discussion.
E	Excellent readability	Age estimates between readers should be the same.

Table 3
Summary data examined otoliths.

Calibration set

Beaufort NC Otoliths from 100 fish, randomly selected from collections made in 1984. Mostly whole otoliths with few sections from older fish. Age range 2-15 years.

Panama City Otoliths from 203 fish, randomly selected from collections made from January through Dec. 2000. Mostly whole otoliths with few sections from older fish. Length range was 462-1300 mm FL. Age range 1-28 years.

MARMAP Otoliths from 100 fish, randomly selected from collections made in March and April 2005. All were whole otoliths. Total length from 591 to 1134 mm and age range 1-14 years

Whole/section comparison

MARMAP Otolith from 100 fish, one whole and one sectioned. Otoliths were selected from collections made from January, February, March, July, and August of 2005. Total length 591 - 1134 mm and age of 3-15 years.

Table 4

Summery of calibration readings

	APE	Average CV
all	5.45	7.13
MB-GF	4.90	6.92
MB-MR	3.62	5.11
MB-JP	2.71	5.74
GF-MR	4.48	6.34
GF-JP	2.31	3.27
MR-JP	3.90	5.51

APE: Average Percent Error, CV: Coefficient of Variation
Readers: MB: Michelle Bahm (MARMAP), GF: Gary Fitzhugh (NFMS-FL), MR: Marcel Reichert (MARMAP), JP: Jennifer Potts (NMFS-NC).

Table 5

Summary of whole/sectioned comparisons

	APE	CV
MR	5.16	7.30
MB	4.03	5.70
GF	2.87	4.06
JP	2.61	3.69
SM	2.74	3.88

APE: Average Percent Error, CV: Coefficient of Variation
Readers: MB: Michelle Bahm (MARMAP), GF: Gary Fitzhugh (NFMS-FL), MR: Marcel Reichert (MARMAP), JP: Jennifer Potts (NMFS-NC), SM: Stephanie McInerny (NMFS-NC).

Figure 1
Reader bias plots for results of the calibration readings. Error bars are ± 1 SD. Reader codes are given in Table 4.

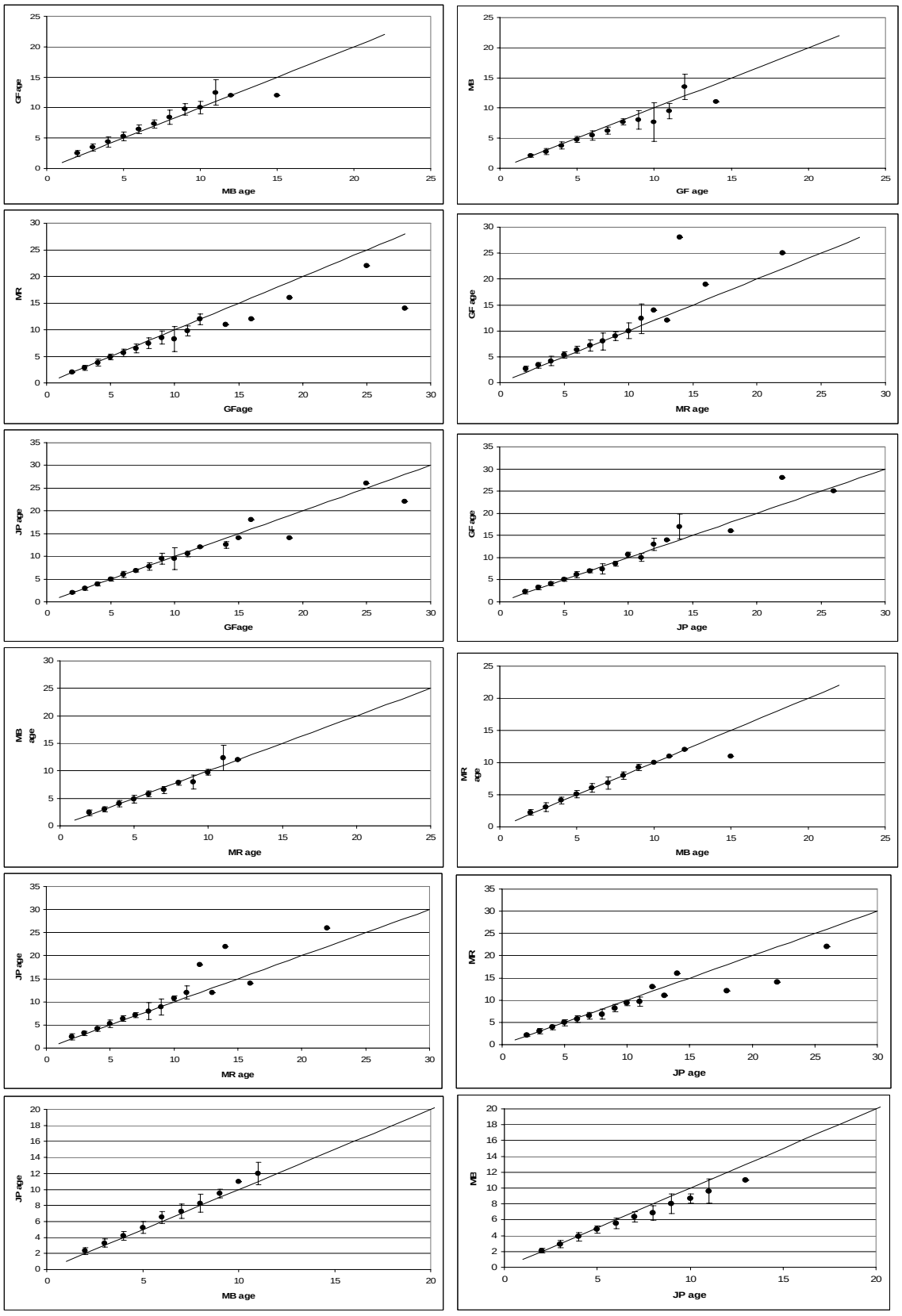

Figure 2
Reader bias plots for results of the calibration readings. Error bars are ± 1 SD. Reader codes are given in Table 5.