FIRST DRAFT

The use of an otolith reference collection to monitor age reader precision for red grouper (*Epinephelus morio*)

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Introduction

The National Marine Fisheries Service Panama City Laboratory has been in operation for 40 years. During that time the laboratory has provided age data for various fish species and coastal sharks for stock assessment purposes. An archive of over 250,000 hard parts including mostly sagittal otoliths, spines, rays, and scales continues to grow and provides additional data for numerous statistical models.

Species-specific age data are required for the Southeast Data Assessment Review (SEDAR) process. In July 2006, SEDAR 12 will commence to discuss the red grouper, *Epinephelus morio*, stock assessment issues with age data provided by the Panama City Laboratory. To insure the quality of the age data provided and to meet the appropriate standards, a red grouper otolith reference collection was created to monitor precision among four otolith readers. These four readers are responsible for completing age determination of red grouper collected in 2002 – 2005 that will be used by SEDAR 12.

The importance of this study is twofold: (1) to monitor in-house reader precision and (2) to insure that quality age data are being generated for stock assessment needs. Previous studies (e.g. Allman, 2004) demonstrated that the use of a reference collection is an ideal tool for validation of ageing methods. The primary role of an otolith reference collection is to monitor ageing consistency for the short-term and long-term duration of a reader's tenure (Campana, 2001). Our objective for this study is to determine if an otolith reference collection is an acceptable means to monitor reader precision among four readers who will be providing age data for SEDAR 12.

Methods

Training readers

The sagittal otolith as described by Moe (1969) was used as the ageing structure for the red grouper reference collection. Prior to reading the reference collection a training set made up of sectioned and whole sagittae was developed. The training set

otoliths were selected to illustrate the various edge types and growth increment patterns typical in red grouper otoliths. L. Lombardi-Carlson is the only reader involved with this study that has previous experience ageing red grouper. Because of that fact L. Lombardi-Carlson was designated as the primary reader. Three new red grouper otolith readers (R. Farsky, C. Gardner, and C. Palmer) first reviewed and aged the training set as a group then aged the training set individually.

Reference collection

A total of 240 sagittal otoliths were selected from the NMFS Panama City Laboratory red grouper otolith archive representing the years 2002 – 2005. Sixty otoliths were selected from the combined four quarters of all three years to insure that a good mix of seasonal annulus growth patterns were present in the otoliths (Figure 1a). The fork lengths of the red grouper to be aged in the reference collection ranged between 354mm and 863mm (Figure 1b). An ad hoc selection of otoliths insured that the reference collection represented a mix of easy, hard, and difficult to read otoliths as suggested by Campana (2001).

Otolith interpretation

Otoliths were read using a stereo microscope with magnifications between 5.625x and 75x. Those otoliths that could not be read whole (band formation could not be interpreted with confidence) were sectioned using the methods as described by Cowen et al. (1995). Annuli were defined as opaque bands, and readers recorded the number of annuli and edge type (Lombardi-Carlson et al. 2002). The four readers involved were provided with only a vial or slide number (as labeled per otolith) and no meristic data were available. Age data (band count, edge type) was entered into a Microsoft EXCEL™ spreadsheet for analysis.

The edge type, band count, and capture date were used to calculate the annual age of a fish based on calendar year (Jerald, 1983). Three different classifications for edge types were used to determine if annual ages were advanced one year. Otoliths with a

complete opaque zone on the edge of the otolith were classified as edge type 2. The number of annuli and the annual age are equivalent for edge type 2. Red grouper undergoing new growth have varying degrees of translucent growth zones on the edge of the otolith. If translucent growth was less than one-half complete, the otolith was classified as edge type 4, and the number of annuli was the same as the annual age. Edge type 6 had a complete translucent growth zone on the edge, and fish age was advanced one year if the fish was captured prior to July 1st. See Table 1 for the criteria for advancing ages.

To insure ageing consistency between readers, and to validate ageing methods, the percent occurrence of opaque edges by month per reader was calculated. This was accomplished by plotting all edge type 2 otoliths per reader by month to insure that band deposition was occurring during the same time of year as noted per reader.

Precision

Four indices of precision were used to determine the level of accuracy between readers. Average percent error (APE), coefficient of variation (CV), and precision (D) were calculated for pairs of readers (Kimura and Anderl, 2005; Campana, 2001). Additionally, percent agreement (PA) was determined and used as a reference to compare agreement (e.g. ± 1 or 2 bands) of ages between pairs of readers. Any large disagreement (e.g. ± 5 bands or more) in ages of otoliths were re-read to check for a transcription or data entry error. A series of paired reader age bias plots was reviewed to check for any ageing inconsistencies.

Results and Discussion

The review of the training set provided an ample representation of the growth patterns in red grouper otoliths. We agreed that after ageing the training set as a group and individually we were confident enough to read the reference collection. There were no indices of precision calculated from the individual readings of the training set.

The reference collection was composed of ages 3 – 23 as aged by the primary reader (Figure 1c). The annual peak of opaque band deposition occurred in May (Figure 2). Percent opaque edges (edge type 2) illustrates that reader C. Gardner shows a higher peak for annulus formation in the month of August versus the three other readers. However, this would not affect the assignment of annual ages of those fish because the edge types represented here are only edge type 2 otoliths and are not advanced (Table 1).

Paired reader bias plots (Figure 3) show no significant bias up to age 12 in any of the paired readings. After age 12 the standard deviation of the ages increases slightly. Lombardi-Carlson et al. (2002) determined that red grouper were fully recruited to the fishery by age 5 – 7 and were not as common by age 12. We suggest that the increase in the standard deviation of the paired reader bias plots was a result of a low sample size in the reference collection of those rare older fish (Figure 1c). Our age bias plots do not reveal any systematic errors between readers.

Overall reader pair comparison results show high precision between all four readers. An APE of 3.45%, CV of 4.28%, and a resulting 2.27% index of precision (D) reflect low reader error. Percent agreement reader comparisons of the primary reader and between all secondary readers show the overall agreement was 100% up to ± four bands (Table 2 and Table 3). Thus, we suggest that the use of a reference collection is an acceptable means to monitor precision between readers. Our overall results suggest that the age determination of the four readers were reliably consistent for the 2002 – 2005 red grouper age samples.

References

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Table 1
Criteria for advancing ages

Collection date	Edge type	Advance annulus count
January 1 – June 30	2, 4	0
January 1 – June 30	6	+1
July 1 – December 31	2, 4, 6	0

Table 2
Reader pair comparison APE, CV, and D

Reader pair	APE	CV	D
L. Lombardi-Carlson – R. Farsky	2.51%	3.55%	2.51%
L. Lombardi-Carlson – C. Palmer	2.87%	4.07%	2.81%
L. Lombardi-Carlson – C. Gardner	1.82%	2.57%	1.82%
R. Farsky – C. Palmer	2.55%	3.61%	2.55%
R. Farsky – C. Gardner	2.21%	3.13%	2.21%
C. Gardner – C. Palmer	3.02%	4.28%	3.02%
Overall	3.45%	4.28%	2.27%

Table 3
Reader pair PA

Reader pair	± 0yr	± 1 yr	± 2yr	± 3yr	± 4yr
L. Lombardi-Carlson – R. Farsky	66%	91%	98%	98%	99%
L. Lombardi-Carlson – C. Palmer	64%	90%	94%	98%	100%
L. Lombardi-Carlson – C. Gardner	72%	94%	94%	98%	100%
R. Farsky – C. Palmer	68%	89%	95%	97%	100%
R. Farsky – C. Gardner	71%	91%	96%	99%	100%
C. Gardner – C. Palmer	65%	88%	93%	97%	99%
Overall	68%	91%	96%	98%	100%

Figure 1. Description of red grouper reference collection, (a) sample size by quarter of year, (b) length frequency, and (c) age frequency of primary reader (L. Lombardi-Carlson).

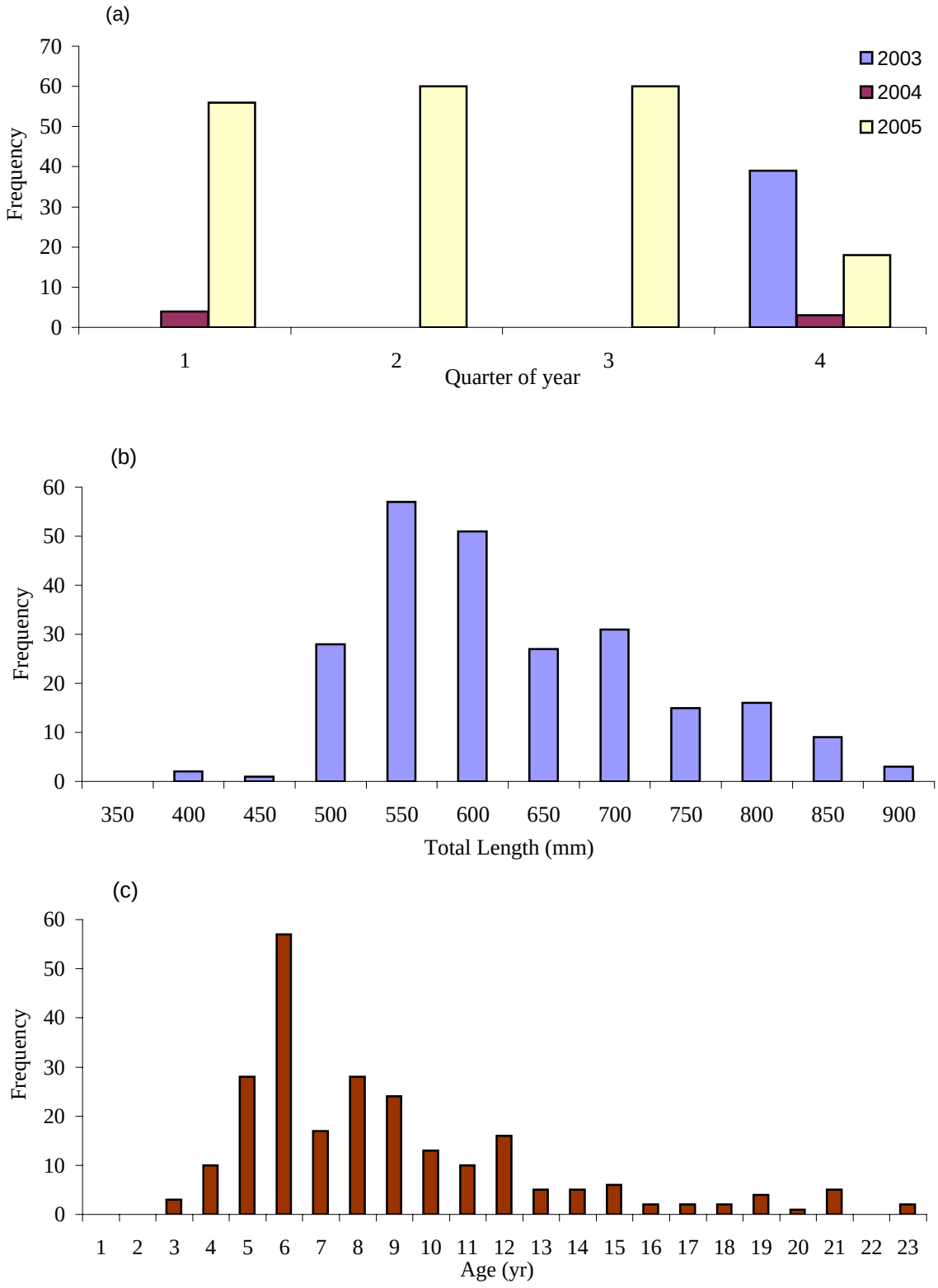

Figure 2. Percent opaque edges by month.

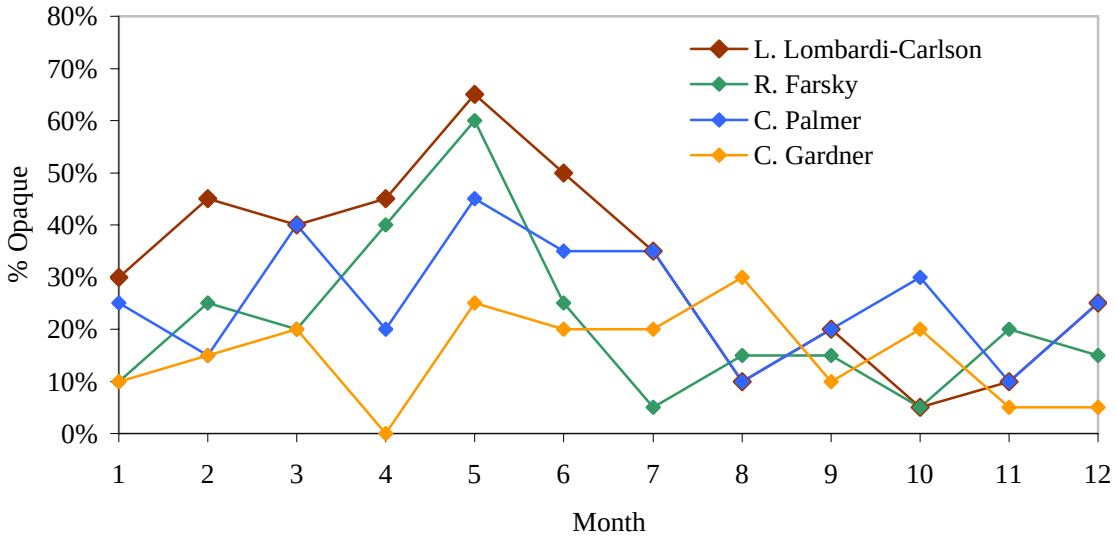

Figure 3. Reader age bias plots by primary and secondary readers. Error bars are +/- 1 standard deviation.