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Jessica Carroll, Kristen Rynerson, Brittany Barbara

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Accuracy and precision of Yellowtail Snapper (*Ocyurus chrysurus*) age determination

JESSICA CARROLL, KRISTEN RYNERSON, BRITTANY BARBARA

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Florida Fish and Wildlife Conservation Commission

Fish and Wildlife Research Institute

100 Eighth Avenue Southeast

St. Petersburg, FL 33701

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Introduction

The Southeastern US Yellowtail Snapper, *Ocyurus chrysurus*, stock is being assessed through the South East Data, Assessment, and Review (SEDAR 64) process. Yellowtail Snapper is a shallow-water-tropical and sub-tropical reef-associated species with a distribution that ranges throughout the Western Atlantic from North Carolina to Brazil (Garcia et al. 2003, Allman et al. 2005). The majority of the fishery for this species occurs off Southeast Florida and the Florida Keys (McClellan & Cummings 1998). The Florida Fish and Wildlife Conservation Commission (FWC) is leading SEDAR 64, and previously led Yellowtail Snapper stock assessments SEDAR 3 and SEDAR 27A. These previous assessments did not directly report ageing precision metrics. Otolith ages for SEDAR 64 were provided by current and past readers at the FWC's Fish and Wildlife Research Institute (FWRI). Current FWRI staff range from 1 year to 16 years experience ageing. Staff undergo extensive quality control training and review, as these data are vital in understanding the health of fish stocks and facilitate effective fisheries management. The objective of this working paper is to ensure the age data generated for SEDAR 64 met precision standards.

Methods

Processing Otoliths

For age determination, the left sagittal otolith was processed. Otoliths were embedded in a two-part epoxy prior to sectioning. Otoliths were cut using a Buehler Isomet low-speed saw with a multi-blade configuration to create three thin transverse sections (VanderKooy 2009). These sections were adhered to glass slides using a clear mounting medium.

Age Determination

Ages were determined using a stereo microscope with objectives ranging from 0.63X–2.0X magnification using either transmitted or reflected light. Five ageing staff were trained by the primary reader, J. Carroll, using practice materials and an in-house reference collection (calibration set). An otolith reference collection is a useful tool for estimating the repeatability among readers (Campana 2001, Allman et al. 2005).

Each otolith was examined with at least two blind reads. These reads were conducted either by two readers working independently, or by a single reader examining the otolith two separate times. When age estimates did not agree between reads, a third read was conducted to resolve the discrepancy. All ages were determined without reader knowledge of fish length or sex (VanderKooy 2009, Carroll & Lowerre-Barbieri 2019).

Age classes were assigned using annulus count (number of opaque zones), degree of marginal completion (Table 1), estimated time period of opaque zone deposition and date of capture (VanderKooy 2009). This traditional method of assigning ages is based on a calendar year instead of time since spawning (Jearld 1983, VanderKooy 2009). Marginal increment analysis was conducted across several age classes by plotting the percent of otoliths with an opaque zone on the edge by month.

Table 1. Age advancement criteria; margin codes are defined as: (1) opaque zone on edge, (2) translucent zone is less than 1/3 formed, (3) translucent zone is between 1/3 and 2/3 formed, (4) translucent zone is greater than 2/3 formed.

Collection Date	Margin Code	Advance Opaque Zone Count
January 1 – June 30	1, 2	0
January 1 – June 30	3, 4	+1
July 1 – December 31	1, 2, 3, 4	0

Precision Calculations

Precision measurements are valuable for evaluating the structure's ease of age determination, the reproducibility of an individual's age, and the skill level of each reader in a laboratory (Campana 2001). Average percent error (APE) and coefficient of variation (CV) are the two most widely used precision calculations (Campana 2001) and are considered "age independent" methods for determining precision (Kimura & Lyons 1991). APE is calculated as:

$$APE_j = 100\% \times \frac{1}{R}\sum_{i=1}^{R}\frac{\left|x_{ij} - x_j\right|}{X_j}$$

for otoliths with multiple age determinations (*R*), X_{ij} is the *i*th age estimate for the *j*th fish. Disagreement by one year between readers on a 2-year-old fish is weighted more heavily than a one year discrepancy of a 20-year-old fish (Kimura & Anderl 2005). When individual errors are averaged across all samples, the outcome is the average percent error for the data set (Beamish & Fournier 1981, Campana 2001).

CV is the ratio of standard deviation over the mean (Chang 1982) and is written as:

$$CV_j = 100\% \times \frac{\sqrt{\sum_{i=1}^{R}\frac{(X_{ij} - X_j)^2}{R - 1}}}{X_j}$$

A random subsample of all collections was used to determine precision estimates between readers. For SEDAR 64, this subsample of 2,680 otoliths collected from 2014-2017 was used to report precision results. Furthermore, age bias plots of the most recent quality control ageing were generated using FSA: fisheries stock analysis R Package to assess reader precision (Ogle 2018). Both APE and CV were also used to analyze the variability of age data for SEDAR 64.

Results and Discussion

The species-specific otolith morphology of commonly collected snappers allows for proper species identification when data collection, entry, or sample management may have produced errors (Figure 1). FWRI ageing staff are familiar with snapper species common to the state of Florida and recognize mislabeled otoliths during sample processing. Species confirmation during sample processing is the first step of QA/QC.

Examination of otolith edge type supported that opaque increments are formed once annually during late spring (Figure 2). Peak opaque zone deposition occurred during May and June with the highest proportion of opaque zones on the edge (margin code 1) in May at 47%.

Prior to ageing SEDAR 64 samples, training materials were reviewed, and the calibration set was re-read by all ageing staff. The FWRI calibration set spans the entire age structure of the species and is weighted with the youngest and oldest age classes to enable training of proper age determination (Figure 3). During the process of reading otoliths, 23% of Yellowtail Snapper samples were aged by multiple individuals to ensure reader consistency. The randomly selected quality control subsample of otoliths amounted to 7% of SEDAR 64 aged otoliths, and the subsample reflected the age structure of otoliths acquired for SEDAR 64 (Figure 3).

Standard deviation of Yellowtail Snapper ages ranged from 0.221 to 0.728, with an average of 0.421 (Figure 4). Typically, standard deviation increases with age, and the highest precision occurs in younger age classes (Ages 1–6). Surprisingly, the lowest standard deviation was found at age 11, but otherwise the remaining values followed an increasing trend. This general trend is consistent with precision for other species (Palmer et al. 2014, Fitzhugh et al. 2015, Lombardi 2015), and is usually related to reduced readability and/or smaller sample sizes of the otoliths from older individuals.

Campana (2001) suggests an average percent error (APE) of 5% or less as an acceptable benchmark for precision, which corresponds to approximately a 7.6% coefficient of variation (CV) calculation. Table 2 outlines the APE and CV for FWRI Yellowtail Snapper ageing and is well below the reported precision standards. Age bias plots of the most recent quality control ageing (n=1,197) by the six current FWRI readers reveals overall high precision and low bias (Figure 5). No single age class was determined to be significantly different from the consensus age across all readers. Disagreements among younger age classes (ages 1-2) were rare, but when they occurred, the general trend was to over-age the sample. Otherwise, no other bias patterns were notable. Variation amongst the bias plots may be related to reader experience. These Yellowtail Snapper age data exhibit high precision, in part due to the ease of ageing, and can be reliably used for analyses in SEDAR 64.

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Table 2. Overall precision metrics for all FWRI ageing staff

Sample Set	n	APE	CV
Reference Collection	100	3.06	4.03
Quality Control Subsample	2,680	2.93	4.03

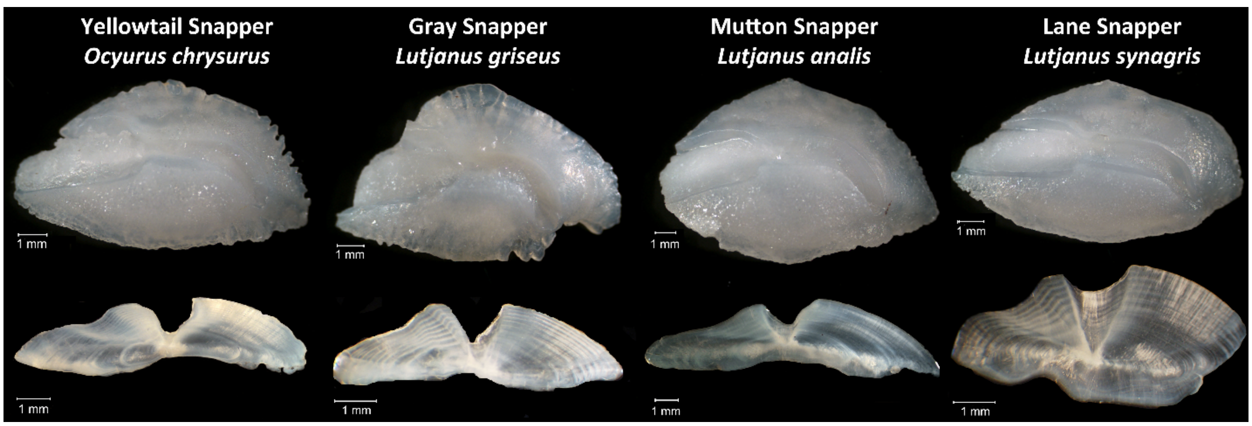

Figure 1. Illustration of the differences between otolith morphology for several snapper species. Differences between whole otoliths of Yellowtail Snapper and other snapper species can be identified by looking at these features: sulcal groove depth (Gray Snapper), overall size (Mutton Snapper), shape and edge smoothness (Lane Snapper). Once processed, variation in sections can be more easily identified. Lane Snapper have deep-bodied sections, Mutton Snapper are proportionally larger, and Gray Snapper have a deep sulcal groove and very distinct opaque zones.

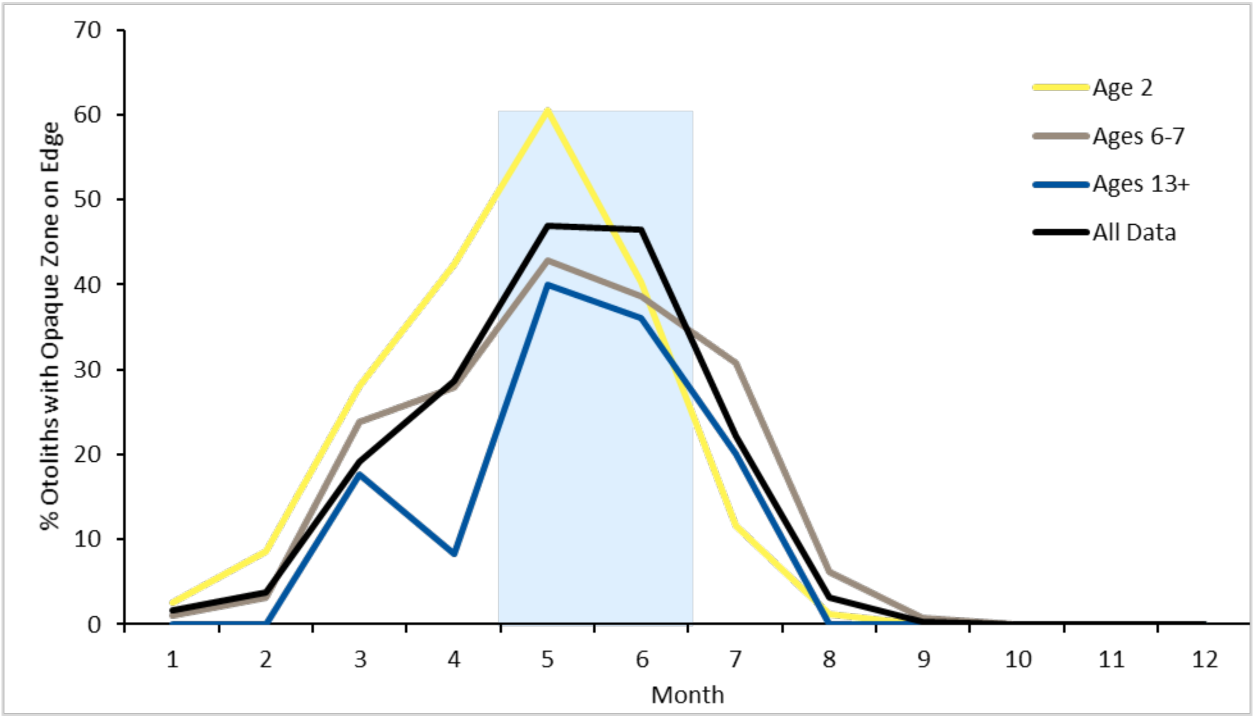

Figure 2. Marginal increment analysis of Yellowtail Snapper illustrating frequency of opaque zone on the edge, by month. Light blue shaded area represents peak opaque zone deposition.

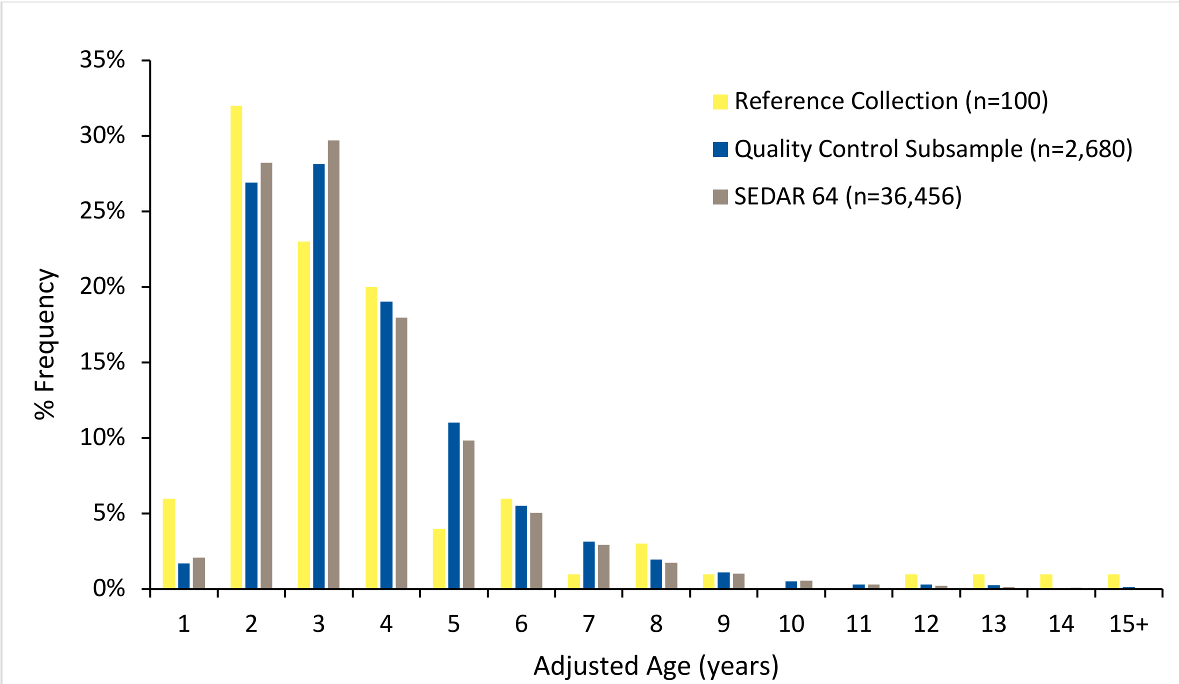

Figure 3. Yellowtail Snapper age frequency for all data, reference collection, and quality control subsamples contributing to SEDAR 64. Reference collection (the internal FWRI ageing calibration set) and the quality control subsample were read by all FWRI ageing staff.

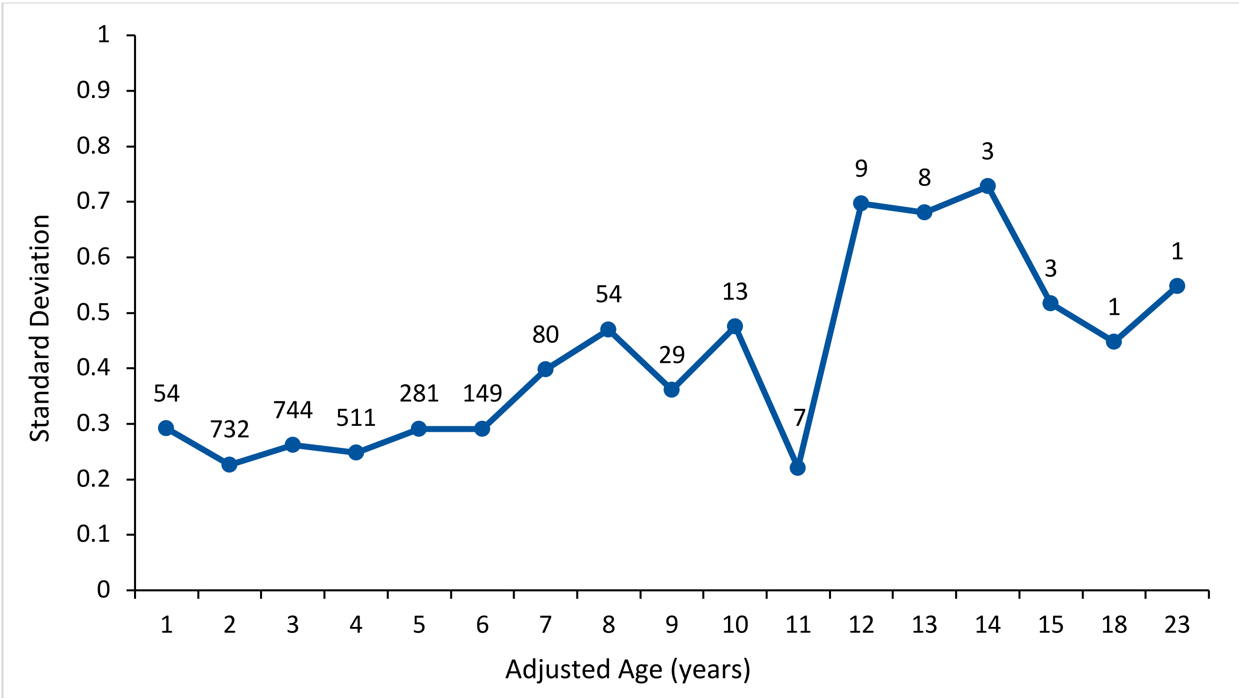

Figure 4. Standard deviation by age for Yellowtail Snapper quality control ageing subsample (n=2,680). Sample size of age classes are shown above each data point.

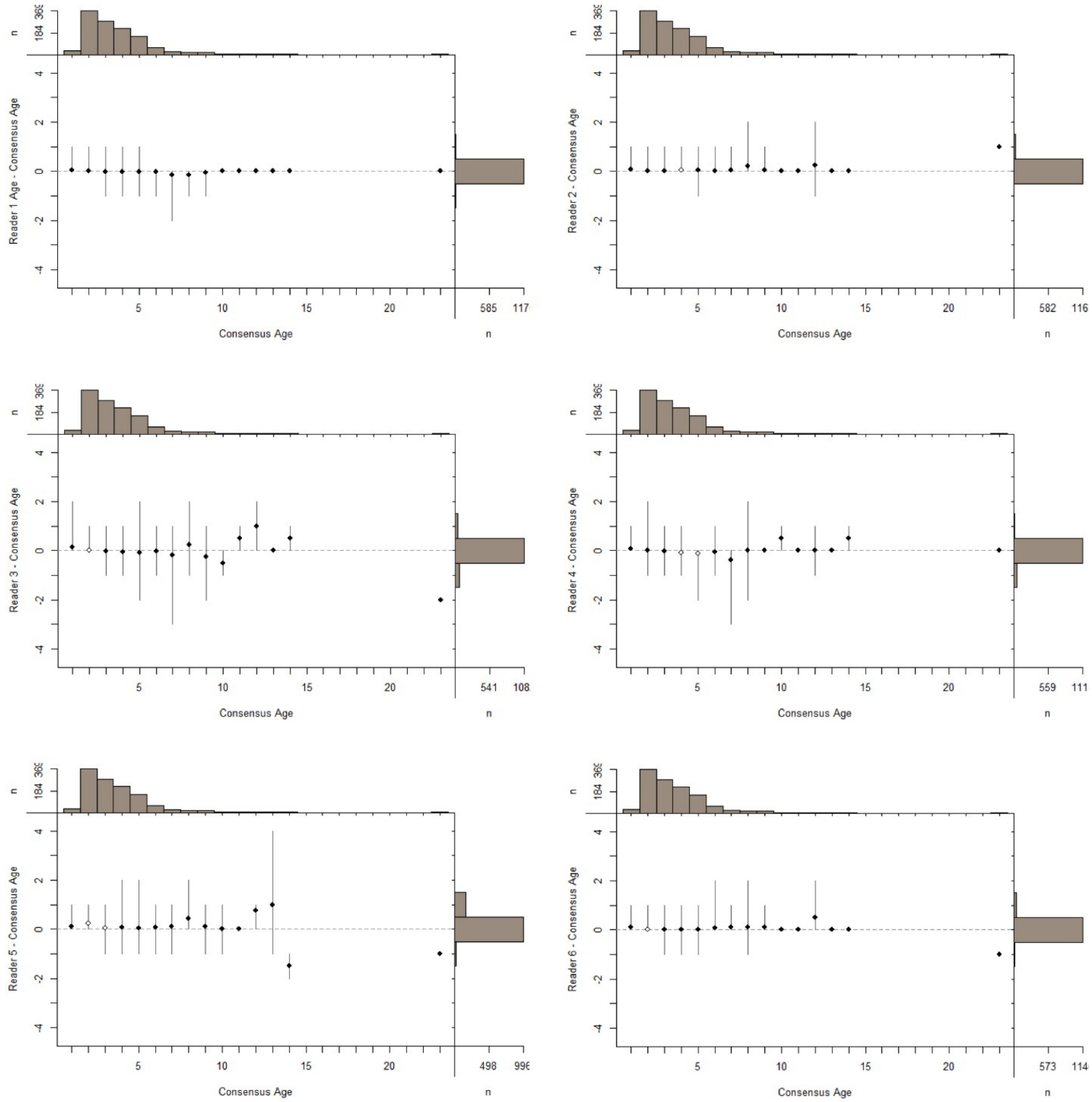

Figure 5. Age bias plots for each current reader of FWRI ageing staff from most recent quality control ageing (n= 1,197). X-axis is consensus age, y-axis is agreement between reader and consensus age, points above or below zero denote over- or under-ageing of the sample, respectively. The vertical lines of each point demonstrate the age estimation range by each reader, and open points indicate when a significant difference was detected between the individual reader and the consensus age. The histogram to the right denotes distribution of age agreement for each reader and the upper histogram illustrates the age distribution of the entire sample.