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Precision of age estimation in red snapper (*Lutjanus campechanus)*

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Abstract

Validation of aging methods, an important step in estimating growth and longevity, has been accomplished for red snapper. However, routine age interpretation remains largely subjective. A reference collection of 300 red snapper otoliths was circulated among 7 aging laboratories in the Gulf region. Average Percent Error (APE; estimate of precision) ranged from 2.5 to 6.0 % for 6 facilities with no apparent bias in estimates as age increased. One initial estimate was notably higher at an APE of 11.6% and bias was evident. For moderately long-lived species such as red snapper, a precision benchmark of ≤ 5% has been suggested. Beyond the need for initial training to recognize annulus patterns in decades-old fish, it was evident that common differences between readers were related to interpretation of the otolith edge type and interpretation of the first annulus. Careful measurement of annulus distances and identification of otolith edge patterns aided by light reflectivity measurements indicated that annual rates of transition from translucence to opacity were fairly consistent. However, annual differences in the seasonal timing of otolith zone transition occurred on the order of a few months (this study compared to others). The degree of opacity varied in the first annulus, and the mean distance from the core to the distal edge of the 1st annulus was 1.05 mm (sd= 0.11). By recognizing possible variations in these factors and with use of a training set and reference collection, our expectation is that a 5% (APE) precision target can be readily achieved and improved upon.

Introduction

Red snapper (*Lutjanus campechanus*) is one of the most economically important fish species in the southeastern U.S. and has been the subject of several age and growth studies (Futch and Bruger, 1976; Bortone and Hollingsworth, 1980; Nelson and Manooch, 1982; Manooch and Potts, 1997; Patterson et al., 2001; Wilson and Nieland, 2001). While validation of aging methods is an important criterion in estimating growth and longevity, and has been accomplished for red snapper (see Baker and Wilson 2001), age interpretation remains largely subjective. Routine annual aging is increasingly performed to track recruitment and age structure trends over time for stock assessment purposes (Allman et al. 2002). Precision of routine age determinations (e.g., from reference samples aged by different readers) ultimately reflects an ability to distinguish strong from weak year classes and is therefore an important concern for assessing stock condition (Beamish and McFarlane, 1995; Crone and Sampson, 1998; Campana 2001).

It has become evident from aging workshops and initial exchanges of red snapper otoliths among laboratories that differences between readers affecting estimates of precision, most often are related to interpretation of the otolith edge type and interpretation of the first annulus (Allman et al. 2002). These two factors have also been noted for other species and are often problematic as standardized age-structure interpretations become more important for production aging (Francis et al., 1992; Fowler, 1995; Campana, 2001). These problems are most acute when errors of one or two years in age-class assignment occur in the most common age classes (i.e., 2-5 years). These errors can mask clear determination of year-class strength—the “smearing effect” (Beamish and McFarlane, 1995).

Our first objective was to compare red snapper ages among 7 gulf laboratories using a reference collection. Secondly, we attempt to resolve the edge and first annulus interpretation problems and to reconcile some potential differences among studies by 1.) determining the timing of opaque zone formation using the method of marginal increment analysis (MIA) based on carefully selected, sectioned and measured otolith samples with broad geographic and seasonal representation (using sectioned sagittae, hereafter referred to as otoliths). We then compare the results from the selected otoliths with those of other red snapper age studies; and 2.) by measuring the otolith core-to-edge distances from seasonally collected juveniles through the period of their first annulus formation. In doing so, we wished to characterize the shape, appearance and location of the year-one annulus.

Methods

Expanded sampling of red snapper otoliths during 1998-2000 in the Gulf of Mexico allowed us to draw a large number of otoliths from all the gulf states and from many different age classes for analysis. Red snapper otoliths were sectioned and mounted for ageing following the methods in Cowan et al. (1995). We designate these as adults even though we later show these otoliths often represent relatively young fish (> 1 year in age). In addition, we were able to obtain a number of young-of-the-year fish from ongoing surveys (NMFS, Mississippi Laboratories) which we designate as juveniles (age ≤ 1 year). These fish were collected on various cruises using trawls of #15 (49 mm stretch) mesh at depths ranging from 16 to 18 meters during the period February 2002 through November 2002. Otoliths were extracted from the juveniles and prepared in a manner similar to the larger specimens but using a low-speed sectioning saw.

<u>Reference collection</u>

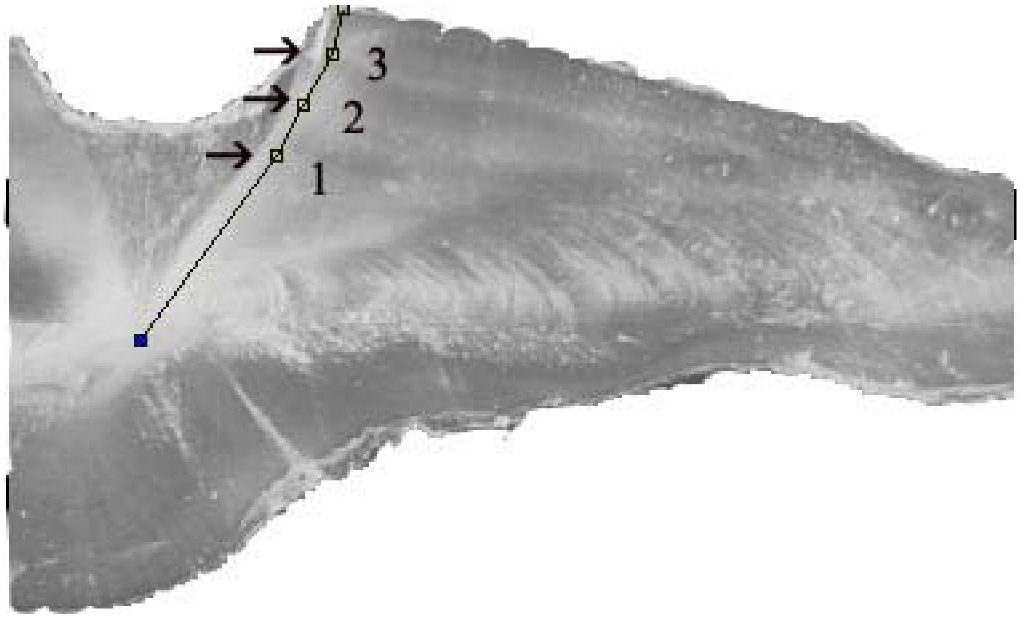
A representative reference set of 300 adult red snapper otoliths was selected from the NMFS Panama City laboratory archive (1998-2000) in order to determine if aging methods among gulf state laboratories were consistent. As has been suggested, the reference collection was selected to represent most age classes, all seasons, both sexes, different collection years, good to poorly prepared otolith sections and the entire geographic range sampled (Campana, 2001). Initially a training CD was created for distribution to all gulf laboratories by photographing and digitizing 100 otolith sections selected from the Panama City reference collection. Each otolith was photographed twice, once without any annotation and once with assumed annuli marked. Otolith training workshops were also held (see Appendix 1 for complete list of red snapper meetings and workshops). Once a general consensus on age assignment was achieved by all laboratories on the training set, the reference collection was exchanged. The reference collection was first used to address within laboratory reader variation, then ages were compared between laboratories. Reference collection ages from 7 outside laboratories were compared to those generated by the Panama City laboratory since Panama City had had notable experience and was initiating the reference collection. Average percent error

(APE; Beamish and Fournier 1981) was used to compare Panama City ages to the other laboratories ages.

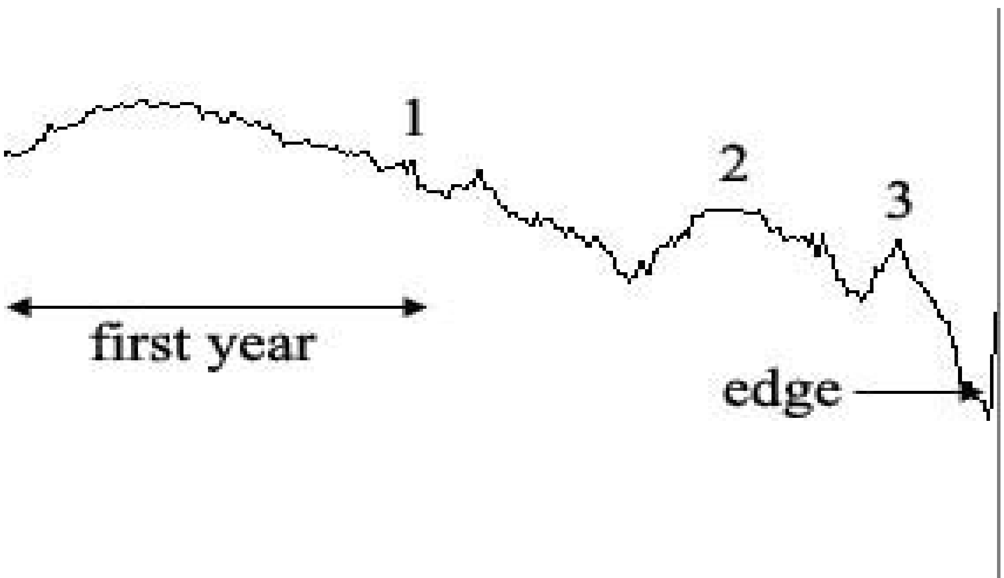
Optical analysis

Over 1000 adult red snapper otoliths were selected from 1998-2000 covering all months and from all five gulf states. From these otoliths, a second selection was made to retain those sections which were precisely cut through the core at an angle perpendicular to the anterior/posterior axis in order to locate a consistent point of origin. We took care to select only those sections for further measurement that did not show edge damage, preparation flaws, or other defects typical of routine age preparations.

Each adult and juvenile otolith section was examined at 40X magnification using reflected light then digitally scanned into the imaging program, PhotoShop 6.0©, equipped with the Andromeda Measurement Filter. The system (camera, adapter, microscope and software) was calibrated using an American Optical Company (1mm/.01mm per division) calibration slide. Earlier work and otolith exchanges had resulted in a presumptive first annulus definition and we enumerated successive opaque zones as annuli following convention (e.g. Baker and Wilson 2001). Measurements to the nearest 0.01 mm were made for each of the following characteristics: core to the outer edge of the presumptive first annulus, core to the center of the second opaque zone, core to center of third zone and any subsequent opaque zone as well as core to edge. Our choice of measurement to the outer edge of the first annulus, rather than the midpoint, was due to the variable and often diffuse appearance of the first annulus (as we define it later). The opaque zones and edges were selected by eye and verified by inspection of the light intensity curves. The measurement path extended from the core to the edge along the dorsal side of the sulcus acusticus (Fig 1 A). The associated light intensity curve represents the red spectra along that path (Fig 1B).



A.



B.

Figure 1. (A.) Measurement path for selected otolith sections and (B.) reflected red spectra light intensity curve for the measurement path. Number 1 indicates the edge and the point of measurement for the first annulus as we define it. Numbers 2 & 3 show peak reflectance for the second and third annuli respectively.

Since we enumerate the opaque zones as annuli, a full annual cycle should include two successive points of maximum opacity (i.e., reflectance). However, the edge distance (marginal increment) was the translucent zone measured from the last opaque zone (maximum reflected light) to the otolith edge. Our preliminary observations revealed that as the translucent marginal increment increased approaching the next season's opaque ring, the reflected light value also increased. Often some opaqueness was apparent to the eye before the annual cycle was complete on the margin. Since the increase in opacity was gradual it was extremely difficult to judge the maximum value until the edge was sufficiently distinct (i.e., opaque) to evidence a decline in light value. Accordingly the edge values were seldom equal to zero.

While marginal increment analysis (MIA) may not be the best method for validation, it does allow insight into timing of otolith zone formation (Campana 2001). Roughly, MIA is divided into edge analysis (frequency of edge type) and an increment measurement approach, the latter being the more common method of MIA (Campana 2001). We will refer to these MIA approaches as edge-frequency and edge-measurement analyses respectively. We employed both an edge-measurement and edge-frequency analysis but compare our findings with edge-frequency results of others. We endeavored to reduce our subjectivity in this approach by careful selection of sections based on orientation and making measurements aided by light reflectivity.

Results

Reference Collection

There were 3 primary red snapper otolith readers at the Panama City laboratory. APE for "in house" readers was 2.5% for the reference collection. Some production aging laboratories consider an APE of 5% as a reference point for moderately long-lived species with relatively difficult to read otoliths (Morison et al., 1998; Campana, 2001). Initial comparisons of Panama City ages to outside laboratories indicated that laboratories 1-4 had an APE below the 5% target (2.8%, 3.5%, 3.7% and 4.5% respectively) while, laboratories 5 and 6 had an APE slightly above (5.9% and 6% respectively). However, major reader differences were noted between the Panama City ages and laboratory 7 with an APE of 11.6% (Fig. 2). To determine if age differences between Panama City and laboratory 7 were systematic, a bias plot was used to compare the mean laboratory 7 age to each of the age categories for Panama City. We noted that laboratory 7 tended to overage younger fish (ages 1-5) and underage older fish (>age 8) (Fig. 3). In contrast, a bias plot of mean laboratory 1 ages compared to Panama City age classes indicated good agreement especially for the most common age classes (i.e., ages< 8) (Fig. 4). Steps were taken to reconcile aging differences between laboratory 7 and Panama City. Subsequent conversation with laboratory 7 personnel revealed that a relatively new otolith reader had done most of the aging. Laboratory 7 also prepared otolith sections using a slightly different method compared to Panama City which might have led to some of the discrepancies. Digitized otolith images were again compared between laboratories. A second reference set of 300 randomly selected otoliths was selected from the laboratory 7 collection and read by both laboratories. An APE of 7.6% was calculated for this collection.

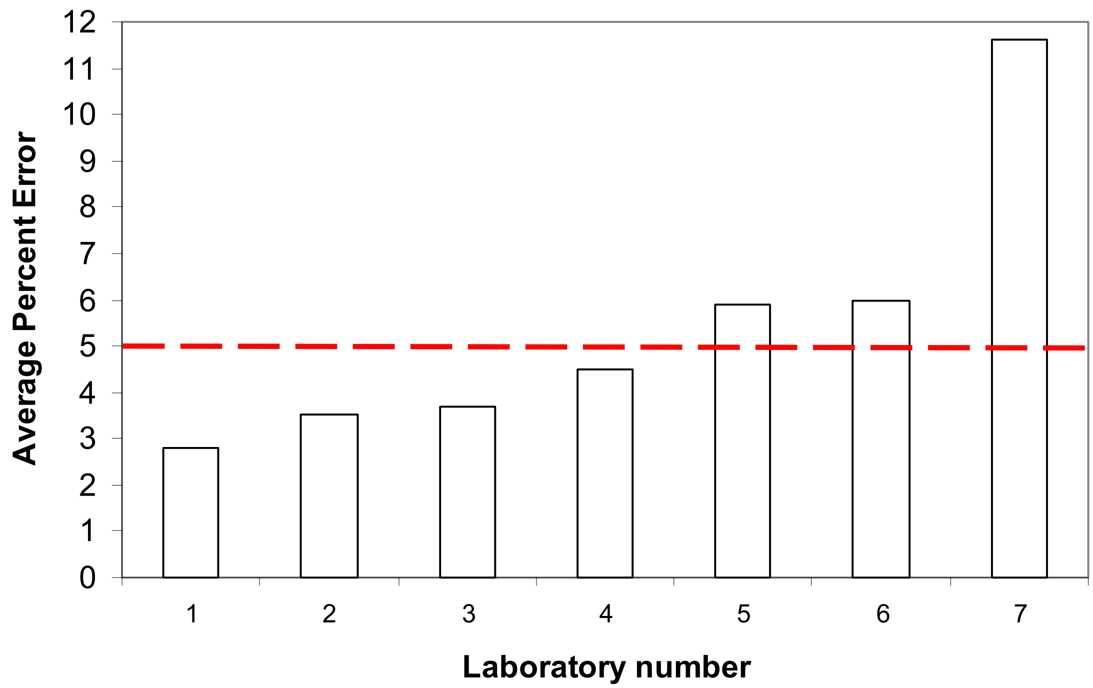


Figure 2. Average percent reader error for Panama City reference collection compared to 7 other gulf laboratories. Dashed line indicates the 5% APE reference point.

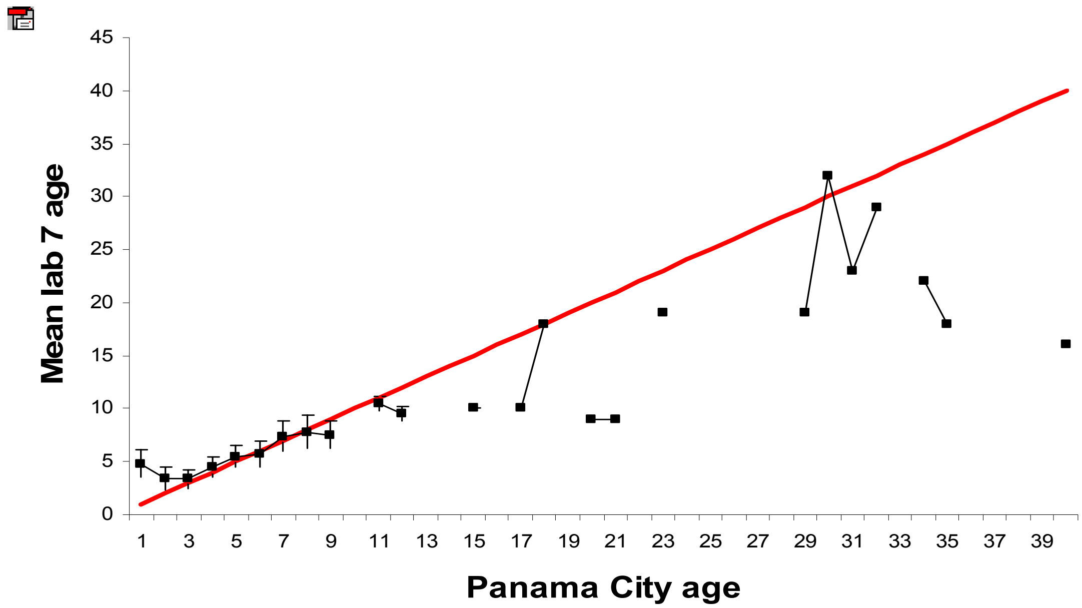


Figure 3. Bias plot of mean laboratory 7 age for each age category of Panama City age ± one standard deviation. Solid line indicates a 1:1 equivalence.

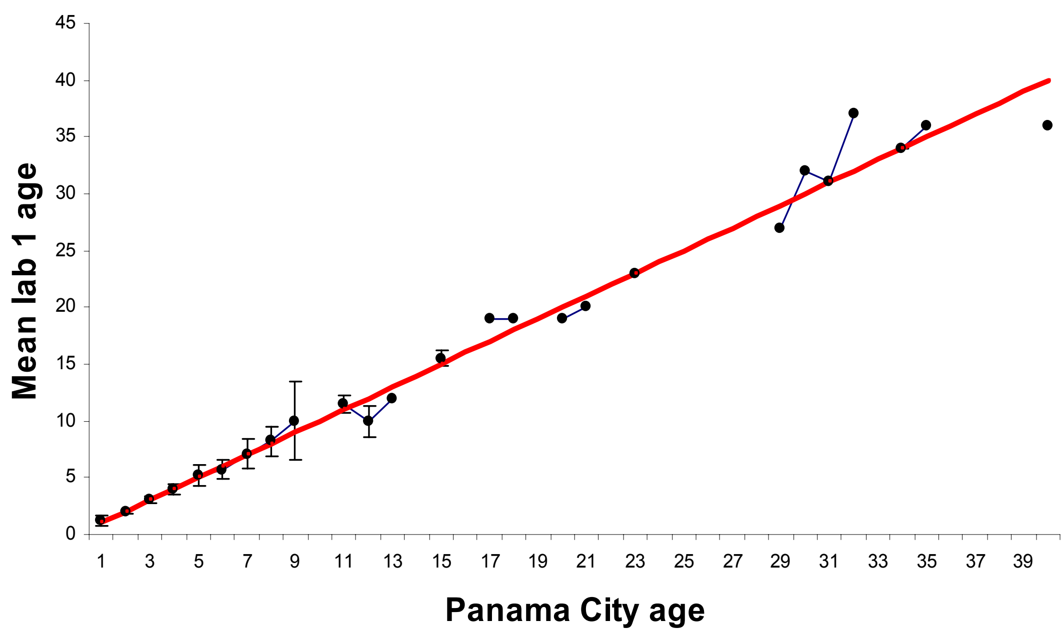


Figure 4. Bias plot of mean laboratory 1 age for each age category of Panama City age ± one standard deviation. Solid line indicates a 1:1 equivalence.

Optical analysis

Two hundred fifty-nine adult red snapper otoliths that had been precisely sectioned through the core were selected, aged and measured. The samples represented all gulf states from 1998-2000 but were obtained primarily from Florida and Louisiana during 1999 (Fig 5A & B). Typical of the age-structure in commercial and recreational fisheries, the fish were young (Allman et al. 2002). Fish having 3 and 4 opaque zones dominated the selected samples (Fig. 5C).

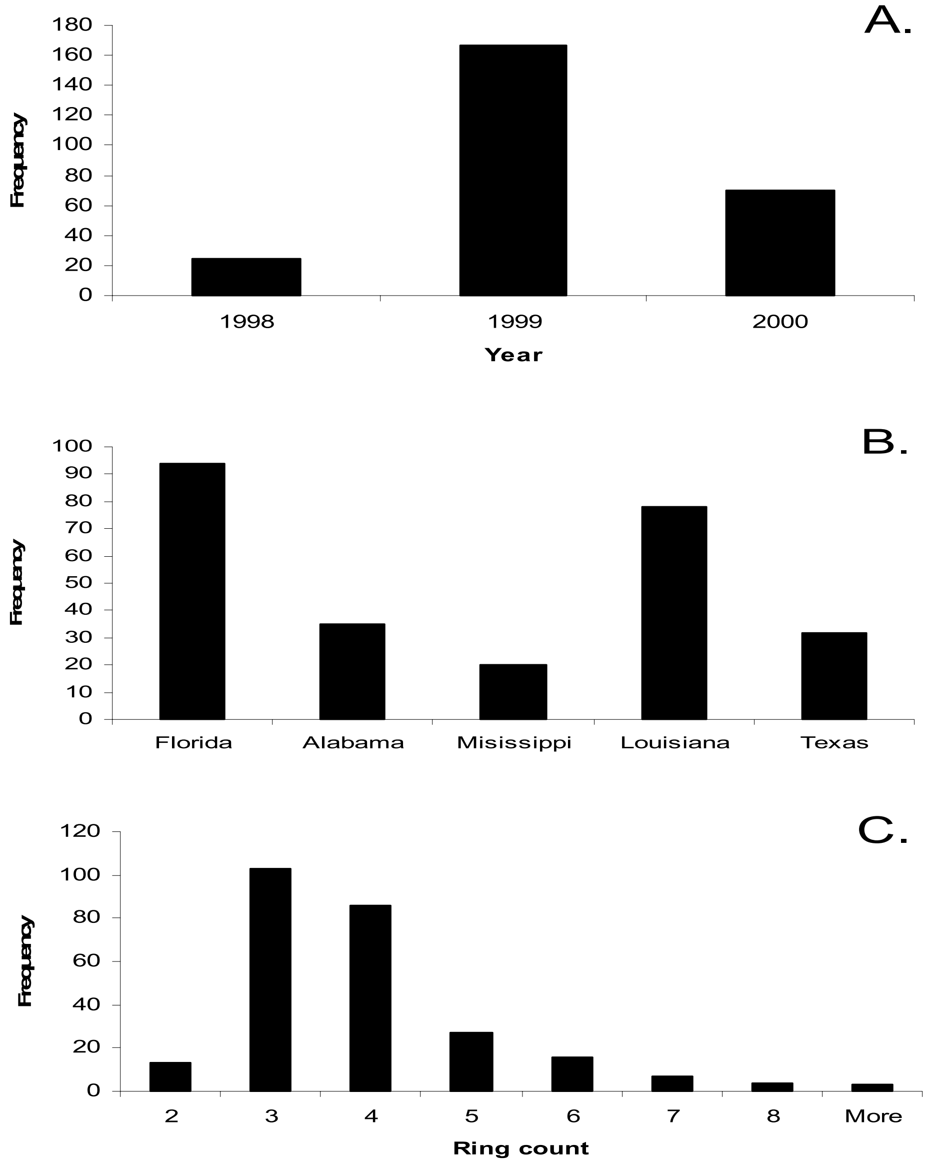


Figure 5. Frequency of adult otolith samples selected for measurement of the marginal increment by A). year, B). state and C.) opaque ring count.

Opaque zone formation

Even with our relatively large sample size used for our initial selection, we needed to combine fishes of different age classes together to have sufficient numbers to examine the seasonal pattern of annulus formation. Therefore, we had to assume that the annual timing of the edge minima is the same for each year class in the subsample. A plot of edge distances against the "month-of-the-collection" indicated that the minimum marginal increment occurred during April through July (Figure 6).

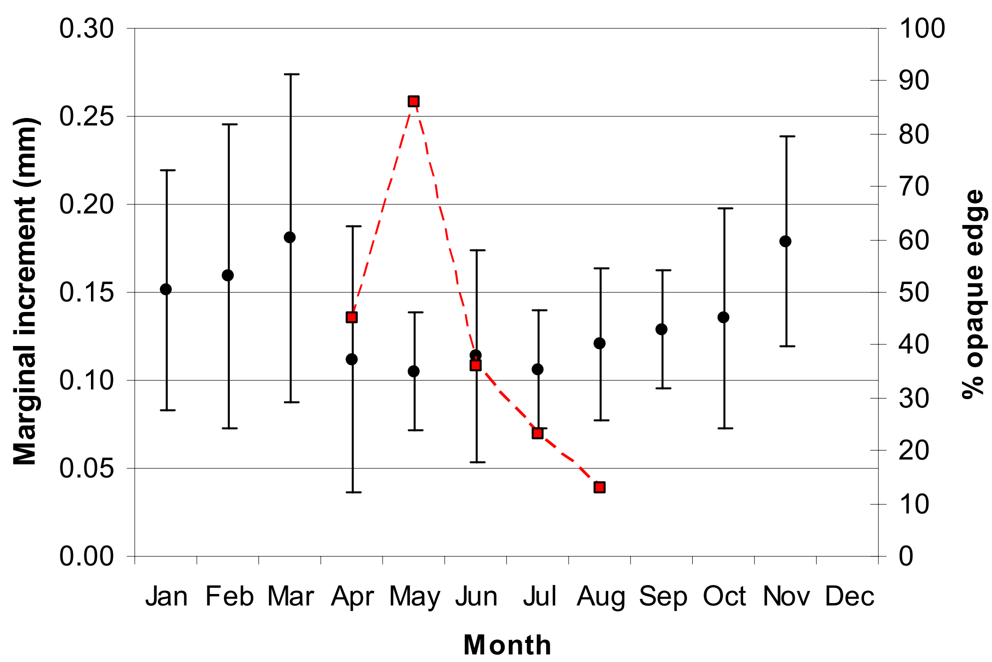


Figure 6. Mean marginal increment (MIA) ± one standard deviation and percent opaque edges (dashed line) by month from archived selected samples.

We also observed that the pattern appeared to be one of rapid increase in opaque zone completion (hence transition to minimal marginal increment) from March through May. When viewed by frequency of opaque edges, peak opacity was observed in May. Opaque zone formation was completed relatively quickly, with frequency declining through August. Marginal increments indicated a gradual increase in the width of the translucent zones from late summer through winter (Figure 6).

<u>First annulus formation</u>

Examination of topologic features indicated that the first year's growth was visually detectable as a broadly diffuse opaque zone of similar dimension among the otolith specimens. This characteristic broad first opaque zone occurred on the dorsal side of the sulcus acusticus (Figures 7 and 8).

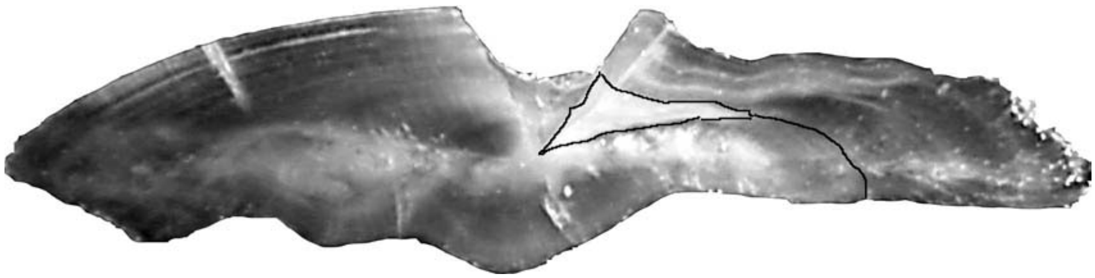


Figure 7. The growth zone of an otolith (outlined by solid line) reflecting a broad first opaque area which includes the presumptive first annulus.

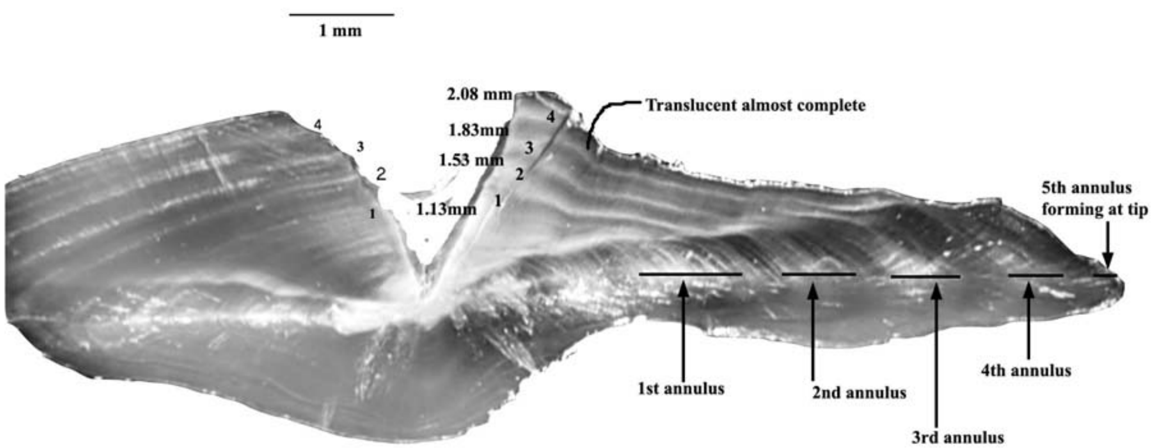
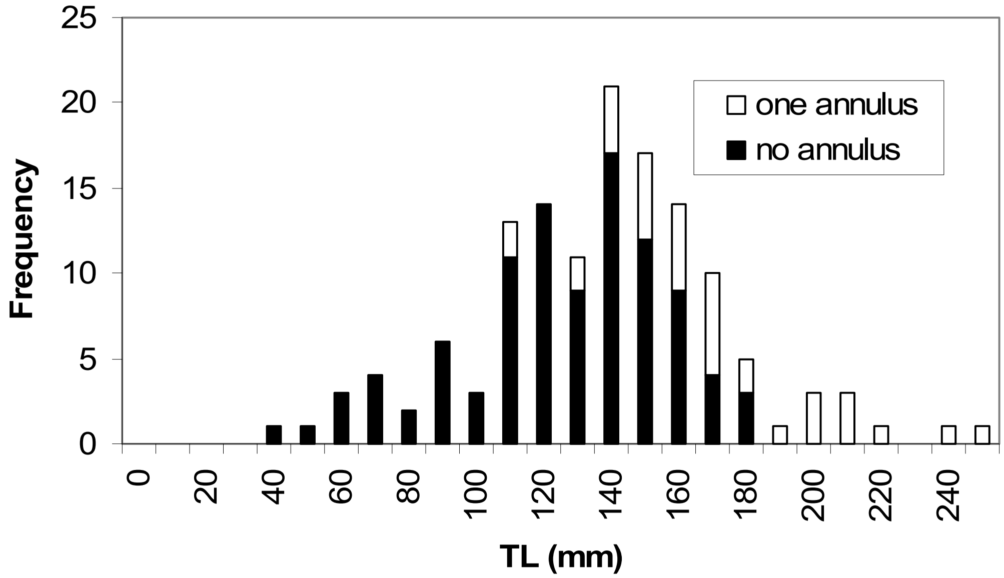


Figure 8. Illustration of the relationship of the first annulus to subsequent annuli. The presumed first annulus is readily evident as a broadly diffuse opaque zone.

To help verify our interpretation, we examined measurements obtained from 136 juvenile red snapper collected through the period of first annulus formation; and ranging in total length (TL) from 33 to 241 mm (Figure 9A).

A.



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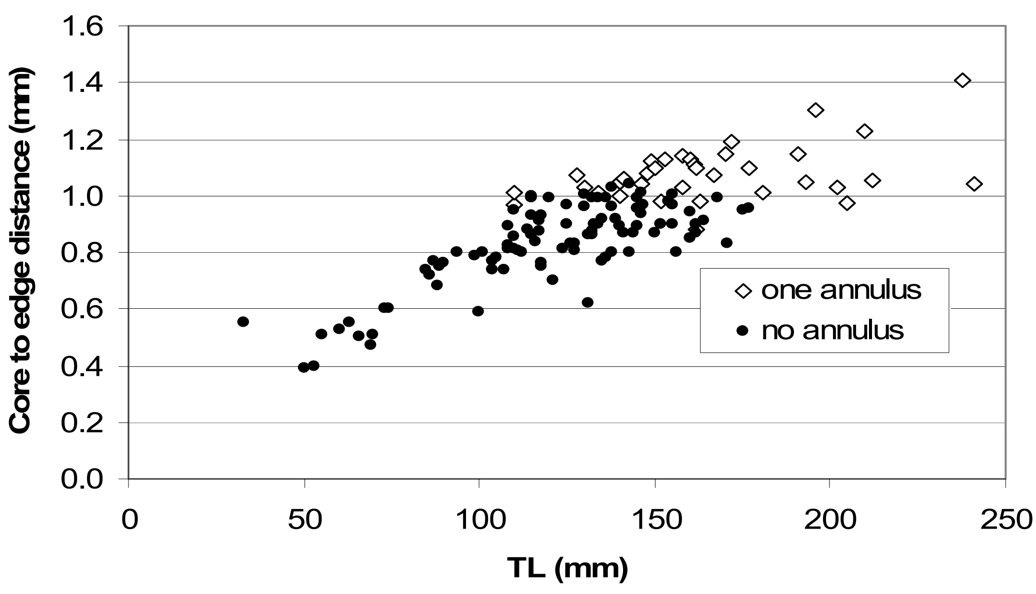

Figure 9. (A.) Total length (mm) distribution of juvenile red snapper examined through the period of presumed first annulus formation and (B.) total length (mm) and distance from the core to the otolith edge for the same juveniles with no annulus and one annulus visible.

Measured core to edge distances were strongly correlated to fish total length, and the edge of the first annulus appeared at a distance of approximately one millimeter from the core (mean=1.05mm, sd= 0.11mm). The first annulus generally formed between 110 and 170 mm TL (Figure 9B). Otoliths from fish having a TL greater than 170 mm were found to have completed the broad first opaque zone.

However, we did observe biological variation (e.g., Figure 10) and during initial routine processing, otoliths were not always sectioned precisely in a transverse fashion through the core. Both of these sources of variation sometimes rendered discrimination of the first annulus difficult.

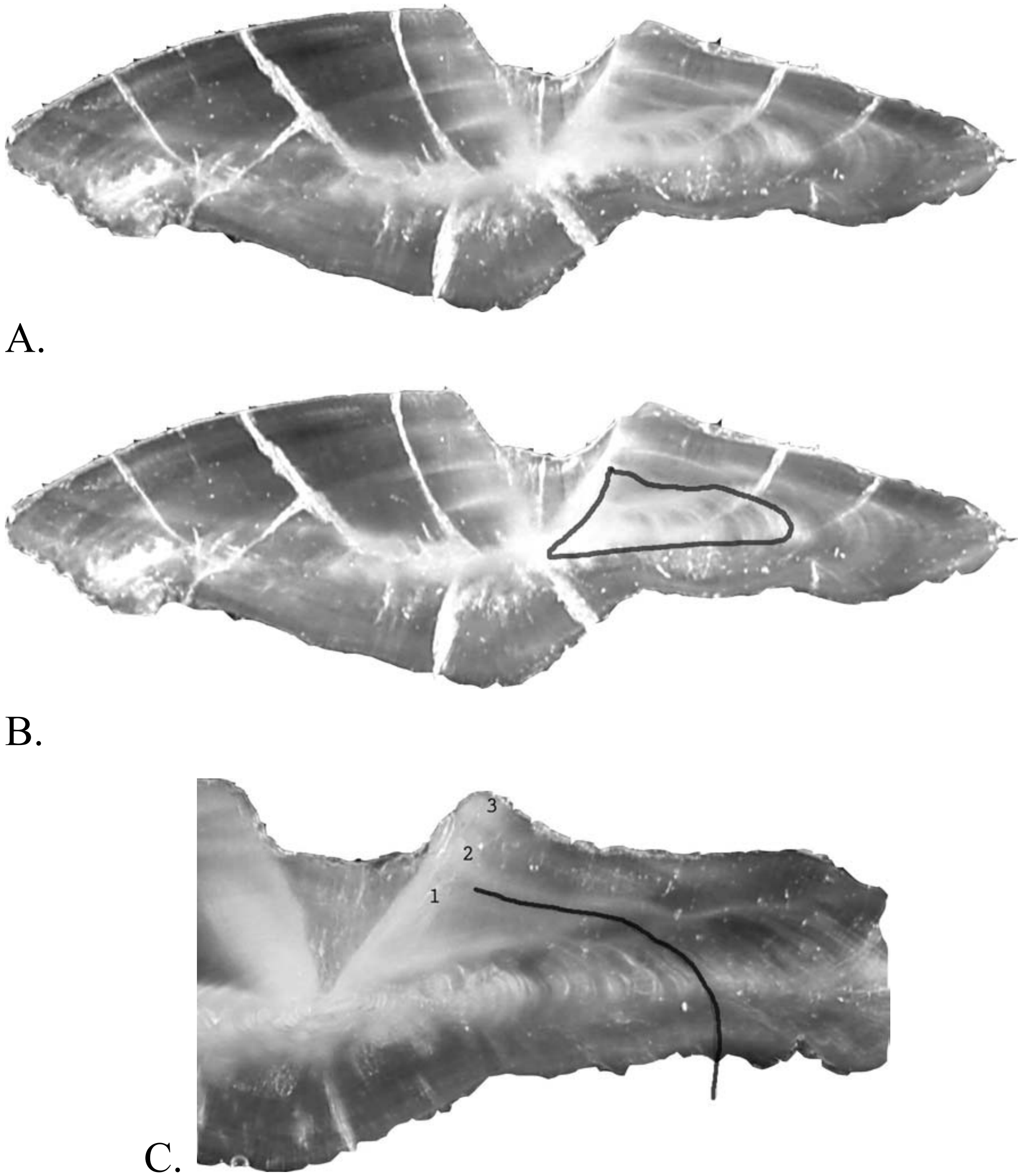

Figure 10. These otolith sections have a count of three opaque zones enumerated as annuli; number three just forming on the edge. The outlined area (Figure 10B) indicates the broad first opaque zone but also shows an area of translucence between the core and the edge of the first annulus (Figure 10A & B are same otolith). This pattern can give the false impression that four annuli are present. The last section (Figure 10C) more closely shows the delineation (solid line) between the first broad opaque zone and the second opaque zone.

Discussion

Reference collection

Inconsistent interpretation is a major problem for production aging laboratories. Red snapper have been relatively difficult to age based on levels of precision determined from this study and from previous studies. This study reported precision estimates of 2.8-11.6% APE for 7 outside laboratories compared to the Panama City reference collection ages. The high value for laboratory 7 (APE= 11.6%) was thought to be due to a relatively inexperienced reader who over-counted annuli in young fish and under-counted the often difficult to distinguish annuli in old fish (i.e., age>8). A second reference collection which was more typical of the ages seen by laboratory 7 (i.e., fewer old fish) yielded an APE of 7.6% when compared to Panama City ages. Other studies have reported precision estimates from 8% to 3.7% APE (Wilson and Nieland 2001, Allman et al 2002). An observed trend for red snapper, commonly seen in production aging laboratories, is that within-laboratory reading estimates were more precise than results obtained from exchanges of otoliths between laboratories (Allman et al. 2002). These results underscore the importance of a reference collection as a crucial quality control tool which must be used through time to insure that individual reader ages do not change over time and that ages from different readers remain consistent (Campana, 2001).

To reduce reader error, improve precision and increase the likelihood that correct ages are assigned, we examined two areas in particular where problems have been noted. These include the timing of opaque zone formation affecting interpretation of the margin and interpretation of the first annulus.

Opaque zone formation

Our edge measurements revealed that there was a relatively sudden appearance of the opaque zone, a minimum marginal increment from April to July, and then a gradual increase in the width of the translucent zone through winter. Our results are broadly consistent with results across taxonomic and geographical boundaries; that is, the opaque zone is complete by spring to summer. The conclusion that this is an annual pattern is also consistent for the juvenile fish we examined. The pattern of sudden transition from translucence to opacity followed by gradual increase in the width of the translucent zone is also consistent with a tropical pattern (Fowler 1995). The opaque zone is thin and the translucent zone is thick. Hence the more visually distinct opaque zone is chosen to be counted as the annulus (Fowler 1995).

The rates of transition from translucent to opaque were fairly consistent (the tropical pattern) for this and for other studies of red snapper (Figure 11). However, we did note that there were differences in the seasonal timing of otolith zone transition on the order of a few months. The only other study performing edge measurements also revealed a sudden formation of the opaque zone, denoted by a minimal marginal increment, but the transition occurred between April and July (Futch and Bruger 1976) as opposed to March and May for this study. By their result, peak frequency of opaque edges would have been expected in July. Several of the studies that utilized edge frequency analysis noted high proportions of opaque edges in February, March and April (Figure 11). Together, these results suggest that the peak occurrence in opaque zone occurrence could range from March through July, while our results show peak opacity occurring during the midpoint of this range (i.e., May). These differences may occur due to interpretation error which is always suspected as MIA is largely subjective (Beckman and Wilson 1995, Campana 2001). In addition, annual or regional differences could have accounted for the shifts in timing of opaque-to-translucent zone formation. Some investigators have suspected or shown evidence that cooler temperatures can delay opaque zone formation (Beckman and Wilson 1995, Pearson 1996, Thomas 1984, Smith and Deguna 2003).

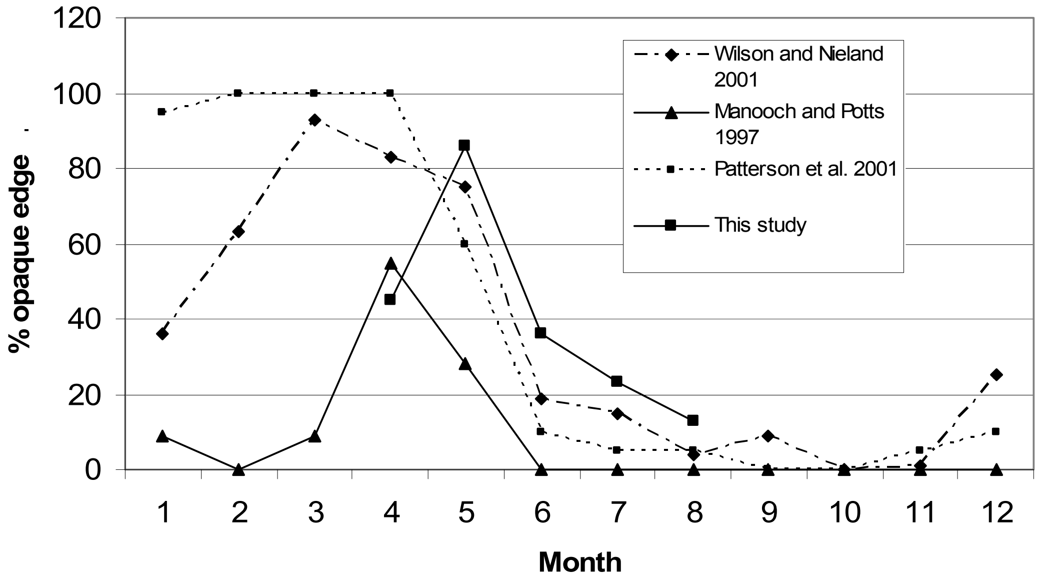

Figure 11. Results of red snapper otolith edge analysis from previous studies. Three studies measured the percent of opaque edges per month. The results from Wilson and Nieland (2001) and Manooch and Potts (1997) were interpolated from graphs similar to that shown. The results from Patterson et al. (2001) were interpolated from a bubble plot and two years were aggregated.

First annulus formation

Red snapper commonly have a broad and diffuse first annulus which has also been noted in other tropical lutjanids and other reef fishes (Fowler 1995). Wilson and Nieland (2001) have hypothesized that a greater distance from the core to the first annulus and the presence of translucence before the first annulus is completed, signal a hatch date early in the spawning season, while an annulus close to the core suggests a hatch date late in the spawning season. In a recent aging manual (Vanderkooy and Guindon 2003) this hypothesis was also repeated to explain the different patterns observed. Among our specimens, we also noted that the degree of opacity varied in the first annulus. Figure 1B shows that reflectivity can be high across a relatively large distance compared to subsequent annuli. This is also confirmed by inspection of Figure 8 and Figure 10C, but these images also show that discriminating the outer edge of the first annulus can be difficult. During reader discussions, it was sometimes thought that the position of the first annulus appeared atypically close to the core, but upon careful examination we decided that this interpretation was incorrect. Consequently, we did not note much variation in the distance from the core to the distal edge of the first annulus when otoliths were carefully sectioned through the core region. Consistently, the distance from the core to the distal edge of the first annulus was about 1 mm.

We compared juvenile fish size at annulus formation with results of other studies as a check on interpretation of the first annulus. We measured the total body length of fish sampled through the spring and summer when they were forming their first annulus based on our interpretation. We noted that the first annulus was forming between 110-170 mm TL at about one year following the expected peak spawning time (convention assumes a spawning date of 1 July; Patterson et al. 2001, based on Collins et al., 1996 and Szedlmayer and Conti 1999). This agrees with previous estimates of size at first annulus; 110 mm TL June and 130 mm TL July modes (Holt and Arnold 1982). In addition the Holt and Arnold (1982) size range of 40-230 mm TL for first-year cohorts sampled from February to December in the 1970s matches our size range for the cohort we sampled over a similar time frame in 2002. Szedlemayer and Conti (1998) surveyed young red snapper using trawl and reported that age-0 red snapper (no annulus observed) ranged to a size of 124 mm SL which is also consistent with the range that we observed.

<u>Implications for precision and accuracy of aging</u>

Knowing when a species is expected to complete an annulus is important in assigning the fish to the correct year class. This is particularly crucial for a production aging approach which is often used to characterize the age structure within a fishery, since fish are often sampled throughout the year. If the timing of annulus completion varies, either geographically or from year-to-year, it can affect the ability to assign the correct age (Smith and Degura 2003, Pearson 1996).

Generally, red snapper follow a typical spring-summer pattern of opaque zone (annulus) formation but timing seems to vary by at least a few months based on the various red snapper studies examined. Currently our aging formula advances fish expected to complete an annulus from the period from January through July which is consistent among all studies. After July, we expect opaque zone formation to be complete and ring count to be equal to age. This is a common approach and one that has been recommended in recent aging manuals (Panfili et al. 2002, Vanderkooy and Guindon 2003). However, the period following expected annulus completion is clearly the period when age assignment is most uncertain (Smith and Deguara 2003) and has been termed the "edge interpretation problem" (Francis et al. 1992). One recommendation may be to target sampling for annual age-structure during the period when opaque zone formation is complete (i.e., late summer through fall). However, restriction of sampling to certain times of year requires the assumption that sampling period represents the age structure for fish collected at other times (e.g., no seasonal change in selection for age).

The often variable appearance of the first annulus has been one of the leading causes of reader disagreements during exchanges of red snapper otoliths. We found size-at-annulus formation to be consistent among studies and this gives us reason to believe that we are in fact interpreting the first annulus correctly. The 1-mm distance (core to distal edge of first opaque zone) may therefore be a good guideline for the expected annulus position and aid in interpretation. A carefully designed otolith marking study of wild juveniles representing a range of spawning dates and possibly incorporating temperature variables, would further help clarify interpretation problems. We feel that making consistent interpretations will elucidate the possible influence of differential spawning time and annual temperature/climatic signals.

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Appendix 1. List of workshops relevant to Gulf red snapper age determination (1999-2003)[1,2]

Gulf of Mexico red snapper aging workshop. January 12-13, 1999. Panama City, Florida. N=13 participants from LSU, USA, NMFS-Panama City, and NMFS-Miami. Topics: Definitions, aging algorithms, training criteria, 1st exchange of red snapper (Box 19).

Gulf-South Atlantic red snapper exchange. February 1999. Beaufort, NC. N=6 participants from NCDMF, SCDNR, NMFS-Beaufort and NMFS-Panama City. Topics: Aging patterns of red snapper and red porgy, 2nd exchange of red snapper (Box 19).

Southeastern reef fish aging workshop, Southern Division Meeting, American Fisheries Society. February 4, 2000. Savanna, Georgia. N=21 participants from SCDNR, LSU, GADNR, UF, ECU, NCDMF, UNCW and NMFS-Panama City. Topics: problem aging of deep water species, red snapper 1st annulus interpretation, vermilion snapper annulus patterns, break and burn techniques, Group comparison aging of 5 species.

Organizational meeting of Gulf otolith processors. April 18-19, 2000. Panama City, Florida. N=18 participants from LSU, FMRI, LADWF, GCRL, MDMR, TPW, ADCNR, UF, GSMFC. Topics: Production aging and need for quality control criteria, age validation, group readings and initial organization of an aging manual[1].

Gulf otolith processors training workshop. July 16, 2002. Panama City, Florida. N=26 participants from GSMFC, MDMR, TPW, LDWF, FMRI, ADCNR, GCRL, UF, NMFS-Beaufort and NMFS-Panama City. Topics: Review of annulus pattern interpretations and group comparison aging of red snapper, greater amberjack, southern flounder, and king mackerel.

Gulf otolith processors training workshop. May 21, 2003. St. Petersburg, Florida. Participants from MML, FMRI, UF, TPW, LADWF, MDMR, GSMFC and NMFS-Panama City, Topics: red snapper quality control and training criteria, reference collection development, comparison aging of several species including red snapper.

[1]In addition to those listed, there were four meetings in 2000-2001, sponsored by the Gulf States Marine Fisheries Commission, specifically to develop an age-structure processing manual for Gulf of Mexico Fishes.

[2]Agencies and Institutions: Alabama Department of Conservation and Natural Resources (ADCNR), Eastern Carolina University (ECU), Florida Marine Research Institute (FMRI), Georgia Department of Natural Resources (GADNR), Gulf States Marine Fisheries Commission (GSMFC), Louisiana Department of Wildlife and Fisheries (LADWF), Louisiana State University (LSU), Mississippi Department of Marine Resources (MDMR), Mote Marine Laboratory (MML), North Carolina Department of Marine Fisheries (NCDMF), National Marine Fisheries Service (Beaufort, Miami, Panama City), South Carolina Department of Natural Resources (SCDNR), Texas Parks and Wildlife (TPW), University of Florida (UF), University of South Alabama (USA).