Analytical Report on the age, growth, and reproductive biology of gag, *Mycteroperca microlepis* from the southeastern United States, 1996-2005.

Marcel J.M. Reichert

David M. Wyanski

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Marine Resources Research Institute
South Carolina Department of Natural Resources
P.O. Box 12559
Charleston, SC 29422

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Contents

1. Introduction

The gag, *Mycteroperca microlepis*, is a large, slow growing protogynous serranid associated with inshore and shelf-break reef habitats in the western North Atlantic from New York to Brazil and in the Gulf of Mexico (Smith 1971, Hardy 1978, Heemstra and Randall 1993). In the South Atlantic Bight (SAB) off the southeastern coast of the United States, gag is commonly collected from sponge and/or coral habitat and rocky outcrops and rocky ledges (e.g., McGovern et al. 1998, Harris and Collins 2000). These areas are patchily distributed throughout the SAB, and patch size can range from square meters to square kilometers (Powles and Barans, 1980). Gag probably make annual spawning migrations to specific locations (Shapiro 1987, McGovern et al. 1998 and 2005, Sedberry et al. 2005).

Gag comprises an important segment of the commercial fisheries in the SAB (Heemstra and Randall 1993, Harris and Collins 2000, NMFS data: http://www.st.nmfs.noaa.gov). In 2003, gag accounted for about 25% by weight, and 31% by value of all serranids landed by US commercial fishermen along the southeast U.S. coast (Figure 1 A-B, NMFS data). Of all species in the snapper/grouper management complex, only black sea bass and vermilion snapper landings exceeded those of gag in recent years (Figure 1A). Since 2000, commercial landings of gag along the Atlantic coast of the southeastern US have been fairly stable between 250 and 275 metrics tons annually (Figure 2), while the value of the catch increased only slightly from $2.50/lb in the late 1990's to $2.80/lb in 2004 (Figure 1A-B). Gag also make up a considerable portion of the recreational harvest of reef fishes along the southeastern U.S. coast (Potts and Manooch 1998, Harris and Collins 2000). In 2003, recreational fishermen reported an annual catch of more than 300 metric tons, exceeding the commercial catches, although the error in the reported recreational catches is relatively large (Figure 2).

Harris and Collins (2000) reported a lower age at first maturity and a significant increase in the observed mean length at age in the SAB gag population in 1994-95 in comparison with data from 1976-82. They concluded that fishing pressure may have been a factor in the described life history changes. McGovern et al. (1998) reported that during the same period the sex ratio decreased from 19.6% males in 1976-82, to 5.5% males in 1994-95 (see also Collins et al. 1987). The size at 50% maturity also seemed to have declined in the later period.

This report documents the analysis of age, growth and reproduction of gag based on collections made from 1996 through 2005 in the SAB. Emphasis was on samples from 2004 and 2005, years in which intensive sampling took place to specifically study maturity, sex ratio, and fecundity. Findings are compared with MARMAP data from the early 1980's and mid-1990's that were reanalyzed.

2. Methods

2.1. Fishery independent (MARMAP) sampling

Fishery independent data collected by the Marine Resources Monitoring, Assessment, and Prediction (MARMAP) program from 1996 through June 2005 were used in this study. Gag were collected during standard MARMAP sampling surveys using chevron traps, hook and line, and vertical longline in the SAB (see Harris and McGovern (1997) and Harris et al. (2004) for descriptions of MARMAP sampling methodology). Specimens were collected during daylight hours between May and September of each year. Buoyed chevron traps were baited with clupeids, deployed from the research vessel, and soaked for approximately 90 minutes. Vertical longline collections were made using a 25.6 meter ground line with 20 hooks baited with squid and set for approximately 90 minutes. Hook and line (rod and reel) collections were made for 30 minutes at dawn or dusk. All collections were made from the *R.V. Palmetto* by MARMAP staff.

Total length (TL in mm), fork length (FL in mm), standard length (SL in mm) were recorded for all specimens using a *Limnoterra FMB-IV* electronic fish measuring board. Total wet weight (FW) was measured using a triple beam balance (± 1g), or a Toledo model 8142 digital balance (± 10 g) for specimens too large for the triple beam, and since 2005 an electronic wave-compensating scale (± 1g, Pols Model P-15). Both sagittal otoliths were removed and stored dry in coin envelopes for later processing. The gonads were removed and a posterior section was preserved in 11% formalin for later histological processing. If the developmental stage of the ovarian tissue was suitable for fecundity analysis (i.e., late developing or ripe with no ovulation), a sample of the ovary was preserved in 10% formalin for later analysis. Seawater was used to make all formalin solutions and marble chips were added as a buffer.

2.2. Fishery dependent sampling

In addition to the MARMAP sampling, gag were also collected from commercial fishermen. Whole gag, caught on commercial snapper reels, were stored on ice shortly after capture and brought to fish houses by the fishermen. Gag were either processed at the fish houses by MARMAP, or state or federal port agents or transported whole to the MARMAP laboratory for processing. For fish processed at the fish house, the sampled specimens were returned to the fishermen for sale (during the regular fishing season) or donated to local charities (during the closed season, see below). In both instances, fishermen were compensated for their efforts.

Published information by McGovern et al. (1998) and Harris and Collins (2000) did not include fecundity estimates since gonadal material was not collected specifically for this purpose. Sampling during 1996-97 and 2000-03 targeted adult females during the spawning season with the primary goal of obtaining gonad samples for fecundity estimates. In 2004 and 2005, a significant effort was made to collect representative samples of gag off the southeastern US (North Carolina, South Carolina, Georgia, and Florida) throughout the year. Sampling during this period included the spawning season closure (March-April) and there was no effort to selectively sample females. During the closure in 2004 and 2005, gag were collected from commercial fishermen under a Letter Of Authorization from the National Marine Fisheries Service (NMFS). Fishermen were asked to collect whole gag of all sizes to a maximum preset number of gag per trip. This trip limit was determined by the port sampling agent conducting the sampling prior to each trip and depended on total number of fish collected to date. Fishermen were compensated for their efforts and all fish that were processed in the fish houses and those processed in the MARMAP laboratory were donated to local charities following the permit requirements.

Total length, fork length, standard length, and total wet fish weight or gutted wet fish weight (±1g) were recorded for all fish. At least one (mostly the left), but possibly both sagittal otoliths were removed and stored dry in coin envelopes for later processing. The gonads were removed, total wet weight (± 1 g) was determined, and a posterior section was preserved in 11% formalin for later processing. If the developmental stage of the ovarian tissue was suitable for fecundity analysis (i.e., late developing or ripe with no ovulation), a sample of the ovary was preserved in 10% formalin for later analysis.

2.3 Age and Growth

Age estimates for gag were obtained from whole otoliths; only those otoliths that were difficult to read or with more than 7 annuli were subsequently embedded and sectioned. However, in 1994 and 1995 all otoliths were sectioned as reported in Harris and Collins (2000). To compare and verify the results of the different methods (whole versus sectioned), otoliths of 100 fish were read both whole and sectioned (see appendix 1 for details).

Sections were taken from the whole left sagittal otolith, which was embedded in an epoxy resin and sectioned transversely, leaving a slice of the otolith with an approximate thickness of 0.5-0.7 mm. This slice, with the core area present, was glued onto a glass microscope slide using Cytoseal. The surface of the preparation was covered with Cytoseal to fill in saw marks in the epoxy resin, which facilitated reading the otoliths. All otoliths were examined by at least two readers who examined each otolith preparation independently and without knowledge of date of collection, size of the fish or other pertinent information. Sections were read using a dissecting microscope and transmitted light. Whole otoliths were read in cedar wood oil (prior to 1994) or water (after 1995) using transmitted and reflected light. During examination of the otoliths the number of increments (counts) was determined, the width of the marginal increment was categorized (1 for opaque zone at edge, through 4 for a wide translucent zone, Table 1) and the quality or readability of the preparation was categorized (A for unreadable, through E for excellent readability, Table 1). Based on previous studies, increments consisting of one opaque plus one translucent zone were assumed to form annually with the opaque zone formation completely formed no later than June 30. In this report, the terms increment count and age will be used synonymously, but note that the increment counts were not converted to calendar age (see appendix I for details). In cases where counts between readers differed, the otoliths in question were read again and examined simultaneously by both readers to reach consensus. Otoliths in the quality category A, and otoliths with persistent count disagreement between readers were omitted from the data analysis.

In September of 2005, readers from the NMFS laboratories in Beaufort, NC, and Panama City, FL, and the MARMAP laboratory held a two day workshop to discuss and calibrate readings of gag otoliths. The results indicated a high degree of agreement between labs and low reader bias (see attached report, Appendix I).

2.3.1. Analysis

Statistical analyses of the sampling, age, and growth data was done with *StatGraphics5-Plus* software. A Von Bertalanffy (VB) growth curve (Von Bertalanffy, 1957) was fitted to the observed counts and lengths using a non-linear regression analysis (Levenberg-Marquardt method). Because of low numbers of collected fish in the MARMAP survey, all years (1996-2005) were combined and the VB parameters were compared with those from previous periods (1986-95, and 1975-85). In the analysis of the fishery dependent sampling, only data from 2004 and 2005 were used. Collections from 1996 through 2003 specifically targeted adult, reproductively-active females for a fecundity study. The age and length structure of this part of the samples was therefore strongly biased. Comparisons of age and growth with data collected previously were done using 2004-05 samples and recalculated data from 1976-82 and 1994-95 (reported in Collins et al. 1987, McGovern et al. 1998, and Harris and Collins 2000). Data were recalculated since additional information, predominantly age, was added to the database after the publications were completed.

Mean lengths, ages, and lengths at age were compared using a t-test with multiple comparisons (α=0.05). If the data indicated a significant departure from a normal distribution, a Kruskal-Wallis test was used. All reported r^2 values in the regression analyses were adjusted for degrees of freedom.

2.4. Reproduction

2.4.1 Maturity and sex ratio

The posterior portion of the gonads was removed from the fish and fixed in 10% formalin for 2-6 weeks, then transferred to 50% isopropanol for 1-2 weeks. Gonad samples were processed with a Modular Vacuum Tissue Processor, vacuum infiltrated, and blocked in paraffin. Three transverse sections (6-8 µm thick) were cut from each sample with a rotary microtome, mounted on glass slides and stained with double-strength Gill's haematoxylin and counter-stained with eosin-y.

Sections were viewed under a compound microscope at 40-400X magnification and one reader assessed sex and reproductive stage using histological criteria (see Table 1 in McGovern et al. 1998), without knowledge of date of capture, specimen length, and specimen age. Because the rate of postovulatory follicle (POF) degradation is a function of water temperature, POFs were assigned

approximate ages according to criteria developed by Hunter and Goldberg (1980) for northern anchovy, *Engraulis mordax*. Gag spawn in outer continental shelf waters with bottom temperatures similar those at which northern anchovy spawn (13-19^{o}C; Hunter and Macewicz 1985). Specimens with developing, ripe, spent, or resting gonads were considered sexually mature. For females, this definition of sexual maturity included specimens with oocyte development at or beyond the cortical alveoli stage and specimens with beta, gamma, or delta stages of atresia. Female specimens with mid to late developing (vitellogenesis through final oocyte maturation), ripe, or spent gonads were considered "definitely mature." To assess the accuracy of our assignments of female specimens to the immature and resting states, length-frequency histograms for immature, resting, and definitely mature females were compared (see also McGovern et al. 1998). Size and age at 50% maturity and at 50% sex transition were estimated with the PROBIT procedure (SAS Institute, Inc. 1989). The LOGISTIC procedure was used to determine which cumulative distribution function (normal, logistic, Gompertz) to use in the PROBIT procedure.

Size/age at maturity and sex ratio were estimated for the 2004-2005 data only, and compared with reanalyzed data from 1977-82 and 1994-95 (McGovern et al. 1998, Harris and Collins 2000). The 1996-2003 collections were excluded since they specifically targeted adult, reproductively-active females, which was expected to highly skew the sex ratio. Transitional specimens were considered males because histological evidence, specifically the narrow temporal window during which sex transition occurs (see McGovern et al. 1998), indicated that they would have been functional males by the next spawning season.

2.4.2. Fecundity

Definitions of total fecundity, batch fecundity, determinate fecundity, and indeterminate fecundity generally followed Hunter et al. (1992). In the present study, potential annual fecundity represented the number of hydrated oocytes matured per year, uncorrected for atretic losses.

Total fecundity: Standing stock of stage-2 and stage-3 yolked oocytes.

Batch fecundity: Number of hydrated oocytes released in one spawning event.

Determinate fecundity: When potential annual fecundity is fixed prior to the spawning season.

Indeterminate fecundity: When potential annual fecundity is not fixed prior to the spawning season.

Three stages of yolked (vitellogenic) oocytes, migratory nucleus (MN) oocytes, hydrated oocytes, and atretic oocytes (*sensu* Hunter et al. 1992) were identified in samples from formalin-preserved gonads. Oocyte size distributions from 20 specimens were used to elucidate temporal patterns in oocyte development. The average radius of each oocyte in a subsample of 150-640 whole oocytes per specimen was determined with Global Lab Image® software and then doubled to get the diameter.

Densities of yolked oocytes from five selected locations in the ovaries of six fish without evidence of ovulation were compared to determine if oocytes were randomly distributed. Two 15-25 mg samples of ovarian tissue, each consisting of 160-280 stage-2 and stage-3 oocytes, were taken per specimen. The effects of location and individual fish on oocyte density were assessed with a two-factor ANOVA without interaction.

A gravimetric method was used to estimate total fecundity and batch fecundity. To estimate total fecundity, two 15-40 mg samples were taken from random locations in 86 ovaries, most (n=60) of which were in the developing stage, and all stage-2 and stage-3 oocytes were counted. We modified the method of Hunter et al. (1992) for calculating total fecundity (TF) by including stage-2 oocytes:

TF = preserved ovary wt (g) * (no. of stage-2 and stage-3 oocytes/sample wt (g)).

Because we did not preserve whole ovaries, fresh gonad weight in reproductively-active females (i.e., those with oocytes undergoing vitellogenesis) was converted to preserved weight with the following equation for (fresh wt = 70-822 g, n = 9, adj. r^2 = 0.999):

Preserved wt (g) = fresh wt (g) * 0.843 + 7.866

The relationship between total fecundity and TL was described for three months (January, February, and April) and the effect of time interval on total fecundity was examined using least squares linear regression and analysis of covariance (ANCOVA).

Gag exhibited evidence of indeterminate fecundity; therefore, estimates of batch fecundity and spawning frequency were necessary to calculate potential annual fecundity. The hydrated oocyte method of Hunter et al. (1985) was generally followed to estimate batch fecundity; modifications were 1) use of a larger sample weight and 2) immersion of samples in a 1-5% formalin solution to enumerate

MN oocytes and hydrated oocytes. Two 40-60 mg samples were taken from random locations in the ovaries of 104 fishery-dependent specimens with MN oocytes and/or hydrated oocytes that were collected during 1996-97 and 2001-05. Batch fecundity estimates were based solely on hydrated oocytes in 103 of 104 specimens. The effect of month on batch fecundity was examined using least squares linear regression and ANCOVA.

Our methods of estimating spawning frequency followed those of Hunter and Goldberg (1980). All females in fishery-dependent samples from 1996-97 and 2001-05 that were reproductively active (vitellogenic oocytes present, developing and ripe reproductive states) were examined for evidence of spawning. Spawning frequency was based on the proportion of specimens with selected histological criteria of spawning (presence of MN oocytes or hydrated oocytes) among reproductively-active females. The proportion was multiplied by the number of days in the spawning season to determine the number of spawning events in that season. The dates of first and last occurrences of spawning indicators (MN oocytes, hydrated oocytes, or POFs) in histological sections were determined to estimate duration of the spawning season. To calculate potential annual fecundity, batch fecundity was be multiplied by the number of spawning events.

All reproduction data were analyzed using *SAS* (SAS Institute Inc. 1989). Results were considered significant at $P<0.05$.

3. Results and discussion

3.1. Sampling, morphometrics, and size frequencies

From 1996 through June of 2005, a total of 2,119 gag were sampled and processed for age, growth, and reproduction analysis. In the MARMAP surveys, the number of gag collected was generally low; a total of 66 gag were collected using primarily vertical longline and Chevron traps (Table 2). The fish were caught between 28.90° N and 34.40° N at depths ranging from 22 to 109 m (Figure 3). The average length of gag from the fishery independent samples was 716 mm TL (n= 65, s.e. 28, range 227 – 1148, Table 4 and Figure 4) and the average weight was 5,842 g (n=64, s.e. 524, range 136 – 17,532, Table 4). Regression analyses indicated the following weight (FW whole wet weight in g) - length (TL in mm) relationship: $FW=3.01*10^{-5}*TL^{2.87}$ (adj.r^2 0.975, Table 4).

The majority of the processed fish (2,053 or 97%) were collected from commercial fishermen in 1996, 1997, and 2000 through 2005 (Table 2). These fish were largely collected using snapper reels between latitude 29.00° N and 33.20° N in depths ranging from 27 to 183 m (provided accurate collection information by commercial fishermen was available). For samples collected in 2004 and 2005, the average length and wet weight were 781mm TL (n=1,598, s.e. 3.4, range 491 – 1,182) and 6,681g (n=1,565, s.e. 94, range 1,390-22,330) respectively (Table 4). Using the 2004-05 data a regression analysis indicated a weight - length relationship of: $FW=1.07*10^{-5} * TL^{3.03}$ (adj.r^2 0.972, Table 4). The length frequency of the gag collected in 2004-05 in the fishery dependent survey indicated a disproportionately large number of fish in the 650 and 700 mm size class (upper limit, black bars, Figure 5 A and B). Also, the very small fish (<500 mm) present in the surveys of 1979-82 are absent in the later surveys (Figure 5 A) due to the implementation of a 20 inch size limit starting in 1991 (Snapper Grouper Fisheries Management Plan, Amendment 4). The presence of a higher number of larger fish in the most recent period of the MARMAP survey can be explained by the introduction of vertical longline gear in 1996 (Figure 4).

The average size of gag collected by MARMAP from the fishery dependent survey was significantly smaller (t-test, p=0.98) than that of fish collected in the fishery dependent survey (Table 4 and Figures 4 and 5). The length/weight relationship in this study is consistent with published data (e.g., Bullock and Smith 1991). The relatively high number of gag in the 650-700 and 700-750 mm TL size classes of the 2004-05 fishery dependent survey may reflect a strong cohort in the actual population or it may be the result of a sampling bias. Since sampling bias may affect the calculations for the sex ratio, we also plotted the length frequency of only the mature fish that we used in the sex ratio calculations. The results indicate that there is no apparent sampling bias in the mature fish (Figure 5 B, see also section 3.3).

3.2. Age and growth

The otoliths of 1,972 gag were processed and examined. Of these, 333 were embedded, sectioned, and read again. Note that 100 of these 333 otoliths were sectioned for a calibration study (age range 4-15, see Appendix I), while the remaining 233 were read sectioned because the readability of the whole otolith was poor or the fish was older than 6 or 7 yrs making increments difficult to discern.

Only 16 otoliths were classified as unreadable. Increments were counted in 57 gag collected by the MARMAP fishery independent survey and in 1,899 fish collected as part of the fishery dependent sampling, of which 1,732 were collected in 2004-2005. The maximum number of increments counted was 25.

3.2.1 Fishery independent (MARMAP) samples

The range of counts of the 57 MARMAP samples collected between 1996 and 2005 was 0 to 15, with an average age of 4.6 (Table 4). A non-linear regression analysis of the increment counts and TL yielded the following Von Bertalanffy (VB) growth parameters: k=0.246 (yr^{-1}), $L_{inf.}$=1074 (mm TL), and t_0=-0.77 (Table 3, Figure 7 A). Relative to the results of the fishery dependent sampling and data provided in the literature, this k value is relatively high, and the L_{inf} relatively low. However, this is most likely a function of a disproportionately large number of smaller fish and a relatively low sample size (n) used in the analysis. With the exception of ages 1, 2, and 7, when the length at age was less in the fishery independent samples, there were no significant differences ($p>0.05$) of the size at age between the samples from the fishery independent and the fishery dependent surveys in the period 1996-2005 (Figure 6A). Analysis of the fishery independent length at age data from 1996-2005 and 1975-85 (insufficient age data available for 1986-95) indicated that all values for 1996-2005 were above those of 1975-85 (Figure 6A). However, only the ages 2, 3, and 4 were significantly different ($p>0.05$), possibly due to low sample sizes.

3.2.2. Fishery dependent samples

The range of increment counts in the otoliths of the 1,899 gag collected in 1996 through 2005 as part of the fishery dependent sampling was 1 to 25 with an average age of 5.6. In 2004 and 2005, the increment counts of 1,632 fish were determined. The average age was 4.52 while the range was 1 – 25 (Table 4). A non-linear regression of the 2004-05 age at length data yielded the following Von Bertalanffy (VB) growth parameter estimates: k=0.186 (yr^{-1}), $L_{inf.}$=1212 (mmTL), t_0= -1.37 (yr) (Table 4 and Figure 7B). The k-values are consistent with the 0.188 given by Harris and Collins (2000), but higher than 0.122 provided by Manooch and Haimovici (1978) for gag in the same geographical area. The values for the L_{inf} were higher than the 1,092 mm reported by Harris and Collins (2000) and the 1,127 mm reported by Manooch and Haimovici (1978). The value of t_0 was consistent with those

reported elsewhere. Recalculations of the VB parameters of the data from the fishery dependent surveys in previous periods (1979-82 and 1994-95) with added available age (counts) data, indicated a slightly lower k value for the 2004-05 data relative to the previous periods (Table 3 and Figure 7D). The analysis also showed an increase in L_{inf} of close to 100 mm TL (Table 4). As expected, a Von Bertalanffy growth curve based on combined data (fishery independent: 1996-2005 and fishery dependent: 2004-05) yielded values for L_{inf}, and t_o close to those of the fishery dependent samples (Table 3 and Figure 7C). However, the value k was notably higher than that of the fishery dependent samples, but considerably lower than that of the MARMAP fishery independent data. These differences can be contributed to the presence of smaller individuals in the fishery independent data set.

A comparison of the size at age for the three sampling periods (Table 5 and Figure 6B) showed that the 2004-05 length at age is significantly larger than that of the 1979-82 collections up to age 12, when sample size becomes very low, reducing the probability of detecting of statistical differences. The 1994-95 data fall between the 2004-05 and 1979-82 data, but size of the younger fish of the intermediate period is closer to that of the 2004-05 data, indicating that the growth rates of gag in these periods is similar and significantly above that of the 1979-82 period (Table 5). However, the size at age of the older fish of the intermediate period is similar to the data from the earlier period. This is explained by the fact that the time period between these sampling periods is about 14 years, and surviving fish with age 1 in the first sampling period would be 15 yrs of age in the second sampling period, following a growth trajectory of the slower growing population 14 years earlier. Similarly, surviving 1 yr old fish from the second sampling period (1994-95) would have been 11 yrs old in the third sampling period (2004-05) and followed the faster growth trajectory (relative to 1979-82). Although not done as part of the current study, back-calculated size at age using the width of annual increments in otoliths of various ages in various years could detail the analysis of growth differences among sampling periods and ages. These findings are consistent with those of the MARMAP fishery independent data and the comparisons of the VB growth curves (Figure 7D), and indicate a shift in size at age of about 15%. This change probably occurred between 1982 and 1995, and only now becomes visible in the older part of the population.

3.3. Reproduction

3.3.1. Maturity and sex ratio

Sex and reproductive state were determined for 99.3% of 1,721 specimens from which reproductive tissue was collected during 2004-05. Correct assignment of reproductive tissue to the immature and resting categories is indicated by the near overlap in the left tail of length histograms for specimens that were definitely mature (i.e., mid to late developing, ripe or spent) and specimens that were resting and by the minimal overlap in the histograms for immature and resting specimens (Figure 8). Specimens of uncertain maturity were intermediate in size between immature and resting specimens (Figure 9).

In the 2004-05 samples, female gag reached sexual maturity at 551-600 mm TL and age 2 (Table 6 and 7). The size ranges for immature and mature females were 491-761 mm TL (median=624) and 588-1151 mm TL (median=828.5). Length and age at 50% maturity were 680 mm TL (Logistic; 95% CI=674-685) and 3.2 yr (Gompertz; 95% CI=3.0-3.3). All females were mature at 801-850 mm TL and age 5. Size at maturity during 2004-05 occurred at noticeably larger sizes than during the intermediate period (1994-95), as $L_{50, Mat}$ increased from 620 to 680 mm TL (Table 8). A comparison of $L_{50, Mat}$ between intermediate and later periods with probit analysis (Normal; L.R. Chi-square, P=0.866, DF=28) revealed that this difference was significant at the P<0.0001 level. This increase in size at maturity had been preceded by a slight decrease in size at maturity between 1977-82 and 1994-95 as reported by McGovern et al. (1998). Age at maturity in the 2004-05 samples was slightly higher (3.2 vs. 2.6 yr) than that for the intermediate period (Table 8); a comparison using probit analysis was not possible, as a model could not be fitted to the data. This slight increase in age at maturity had been preceded by a significant decrease in age at maturity between 1977-82 and 1994-95 as reported by Harris and Collins (2000).

Histological examination of 1128 sexually mature gag collected during 2004-05 revealed that the percentage of males and transitionals has increased from 5.5% in 1994-95 (see McGovern et al. 1998) to 8.2%. The current percentage of males and transitionals is still much lower than the revised estimate of 19.4% for samples collected during 1977-82; McGovern et al. (1998) reported 21.1% males and transitionals in the 1976-82 samples.

In the 2004-05 samples, sex transition occurred during March through July and October through November, with a peak from April through June when 12 of the 17 transitional specimens were captured; total length and age ranged from 828-1104 mm (median=1012) and 3-11 yr (median=8), respectively (Tables 9 and 10). The size and age ranges for male specimens were 805-1182 mm TL (median=1074) and 5-25 yr, respectively. Length and age at 50% sex transition were 1049 mm TL (Logistic; 95% CI=1030-1074) and 9.7 yr (Normal; 95% CI=9.3-10.3). A comparison of these current estimates with estimates derived from earlier data utilized by McGovern et al. (1998) showed that sex transition during three periods has occurred at progressively larger sizes (995, 1024, and 1049 mm TL) and younger ages (11.7, 10.5, and 9.7 yr) since 1977-82 (Table 11); comparisons of these L_{50} and A_{50} estimates using probit analysis was not possible, as a model could not be fitted to the data.

3.3.2. Fecundity

There was no significant difference in the density of yolked oocytes (stage 2 and stage 3) among five selected locations in the ovaries of six specimens (F=0.96, P=0.452, df=4), which indicated that samples for estimating total fecundity and batch fecundity could be taken from any location without bias.

Total fecundity as a function of total length was essentially constant throughout the spawning season and did not exhibit a declining trend over time (Figure 10). Four specimens > 1000 mm TL that were collected in March had noticeably lower fecundity; histological sections revealed the presence of POFs < 48 h old in these specimens and a decision was made to omit March data from the ANCOVA. An ANCOVA showed that the slopes (F=0.39; P=0.681; df=2) and intercepts (F=0.14; P=0.866; df=2) of equations for January, February, and April were not significantly different (Figure 10).

Annual fecundity in gag is indeterminate because total fecundity did not decrease during the spawning season and no size gap between stage-2 and stage-3 yolked oocytes developed at any time during the spawning season (Figure 12). Continuous production of oocytes was also evident, as the percentage of stage-3 yolked oocytes did not progressively decrease over time. To estimate potential annual fecundity, estimates of spawning frequency and batch fecundity are necessary.

Statistically significant relationships were present between batch fecundity and total length, fork length, whole body weight, ovary-free body weight and age (Table 12). Batch fecundity as a function of

total length did not differ between three time intervals (Jan-Feb, Mar, and Apr-May), as indicated by the lack of differences in slopes (F=0.05; P=0.956; df=2) and intercepts (F=2.62; P=0.078; df=2). Given the similarity of the equations, data from all time intervals were combined. Estimates of batch fecundity (BF) for gag 661-1,159 mm TL (BF = -975,902 + ($1.671*10^3$)*TL) ranged from 128,600 to 960,800 oocytes.

To estimate potential annual fecundity (not corrected for atretic losses), batch fecundity was multiplied by number of spawning events. The proportion of specimens with MN (migratory nucleus) oocytes or hydrated oocytes among reproductively-active females was 0.398 (Table 13), which corresponded to an approximate spawning periodicity of 3 d. With a spawning season of approximately 114 d (Jan 13 though May 6) based on the first and last occurrences of spawning indicators (MN oocytes, hydrated oocytes, or POFs) in histological sections, an individual female could spawn approximately 38 times per season. Multiplying the estimated number of spawning events (38) by batch fecundity estimates for gag 661-1159 mm TL (Table 12) produced estimates of potential annual fecundity that ranged from 4,887,900 to 36,509,900 oocytes.

A comparison of batch fecundity estimates from the present study with those for gag in the eastern Gulf of Mexico revealed a moderate degree of similarity, particularly for larger specimens. Collins et al. (1998) reported that the relationship between batch fecundity (BF) and TL (mm) for specimens 690-1065 mm TL was:

$$BF = 1.773 \times 10^3 (TL) - 1{,}119{,}000$$

Estimates of batch fecundity for specimens 690-1065 mm TL ranged from 104,400 to 769,200 oocytes. The BF-TL equation from the present study (Table 12) was applied to the same size range of specimens and resulted in estimates of batch fecundity ranging from 177,100 to 803,700 oocytes.

Our estimate of the number of spawning events (38) per spawning season was notably higher than the estimates of 27, 14, and 8 events during 1991, 1993, and 1994, respectively, for gag in the eastern Gulf of Mexico (Collins et al. 1998). It was very similar to the estimate of 42 spawning events in the 106 day spawning season of a congener, *M. phenax* (Harris et al. 2002).

3.4. Summary conclusions

In summary, both the Von Bertalanffy growth curves and the length at age data from the fishery dependent surveys indicate that the increase in growth rate in the younger age classes between 1979-

82 and 1994-95, did not continue between 1994-95 and 2004-05. However, the older fish in the current population are larger than those of the same age in previous sampling periods. This is due to the fact that the younger, faster growing fish from 1994-95, show up as larger 10+ yr old in the 2004-05 samples.

Size at maturity during 2004-05 occurred at significantly larger sizes than during 1994-95. Age at maturity has also increased, albeit less dramatically than for size at maturity, since 1994-95. These changes are probably connected to the significant increase in growth rate observed at ages 3-5. The percentage of males and transitionals in the population has increased from 5.5% in 1994-95 to 8.2%; however, the current percentage is still much lower than the revised estimate of 19.4% for samples collected during 1976-82. Sex transition has occurred at progressively larger sizes and younger ages since 1977-82, a trend that is also probably related to the increasing growth rates over time. The regression equation relating batch fecundity to TL was reasonably similar, particularly for larger specimens, to one developed for gag in the eastern Gulf of Mexico; however, our estimate of the number of spawning events was notably higher.

MARMAP Project Personnel

Age and Growth: Marcel Reichert, Michelle Bahm, Mark Collins, Patrick Harris,
Reproduction: David Wyanski, Oleg Pashuk, Paulette Mikell
Sample Processing and preparation: MARMAP personnel
Data analysis: Marcel Reichert, David Wyanski, Paulette Mikell, Michelle Bahm, and Jessica Stephen
Report preparation: Marcel Reichert, David Wyanski

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Table 1

Readability and edge codes used in otolith readings.

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 yr 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 yr in young, and within 4 yrs 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 yr for young, to 2 yrs in old fish (> 10 yrs). Agreement after second reading is expected without much discussion.
E	Excellent readability	Age estimates between readers should be the same.

Table 2

Number and source of gag sampled during 1996-2005. FI: Fishery independent sampling (MARMAP), FD: fishery dependent sampling. The numbers between brackets behind the gear indicate the gear code.

	year	1996		1997		1998		1999		2000		2001		2002		2003		2004		2005		total		
	survey type	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	total
gear	Hook and line (14)		1																13		83	0	97	97
	Snapper reel (43)		106		15					1	19		64		66		54		318		1314	1	1956	1957
	Vertical longline (61)							3						2		1				5		11	0	11
	Chevron trap (324)	15		6		4		6		11		4		1				2		5		54	0	54
	total	**15**	**107**	**6**	**15**	**4**	**0**	**9**	**0**	**12**	**19**	**4**	**64**	**3**	**66**	**1**	**54**	**2**	**331**	**10**	**1397**	66	2053	2119
	survey type	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	FI	FD	
	year	1996		1997		1998		1999		2000		2001		2002		2003		2004		2005		total		

Table 3

Von Bertalanffy Growth Parameters for gag (southeastern U.S.)

Survey type	data from	L_{inf} (mmTL)	Asympt.s.e.	k (yr^{-1})	Asympt.s.e.	t_o (yr)	Asympt.s.e.	adj.r^2	n
Fishery dependent survey	1979-82	1096	10.1	0.196	0.006	-1.01	0.02	88.9%	507
	1994-95	1115	10.5	0.200	0.008	-1.62	0.14	71.9%	2268
	1996-05	1210	18.9	0.180	0.009	-1.57	0.15	77.9%	1770
	2004-05	1212	19.0	0.186	0.010	-1.37	0.15	78.7%	1504
MARMAP survey	1976-1985	1142	45.1	0.166	0.016	-1.04	0.18	94.0%	54
	1986-1995	no age data available							
	1996-2005	1074	64.0	0.246	0.045	-0.77	0.26	89.1%	57
Combined data	See text	1184	15.9	0.196	0.009	-1.34	0.12	78.7	1827

Some morphometric and otolith increment data for gag collected off the southeastern U.S. All lengths are in mm and all weights in g. TL: total length; FL: fork length; SL: standard length, WW: whole wet weight; s.e.: standard error around the mean; adj.r^2: adjusted (for degrees of freedom) r-squared; n: number of fish included in the analysis. Note that counts were not converted to age (see text for details).

	Fishery dependent survey 2004-2005				MARMAP survey 1996-2005			
	mean	s.e.	range	n	mean	s.e.	range	n
TL	781.3	3.38	491 - 1182	1598	715.8	28.32	227 - 1148	65
FL	764.4	3.24	476 - 1189	1704	706.1	26.60	268 - 1100	62
SL	686.8	3.29	418 - 1012	1175	600.5	24.36	190 - 978	65
WW	6681	94.42	1390 - 22330	1565	5842	523.8	136 - 17532	64
increment counts	4.52	0.054	1 - 25	1632	4.56	0.358	0 - 15	57
	a		adj.r^2	n	a		adj.r^2	n
FL=a*TL	0.967		99.9	1575	0.967		99.9	62
FL=a*SL	0.843		98.4	1175	0.841		99.5	65
	a	b	adj.r^2	n	a	b	adj.r^2	n
$WW=a*TL^b$	$1.07*10^{-5}$	3.03	97.25	1559	$3.01*10^{-5}$	2.87	97.5	64
$WW=a*SL^b$	$2.45*10^{-5}$	2.98	95.5	1136	$7.11*10^{-5}$	2.82	97.5	64

Table 5

Length at age for gag collected in fishery dependent surveys off the southeastern U.S. in three periods. Increment counts from otolith information were not converted to age (see text for details). Mean lengths given in mm TL. Between parentheses: standard deviation and number of fish (n) used in the analysis. Lengths in bold and indicated with [a] or [b] denote no significant difference. Analyses for fish with counts between 2 and 13 were done using t-tests and multiple range tests (LSD) with differences at the 05% confidence level. Other counts were analyzed using a Kruskal-Wallis test because data were not normally distributed, mostly due to a low n.

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count (yr)	1979-1982		1994-1995		2004-2005	
1	376	(54.2 - 10)	**511**[a]	(21.7 - 12)	**576**[a]	(122 - 2)
2	492	(27.8 - 6)	**585**[a]	(78.2 - 42)	**601**[a]	(53.5 - 58)
3	583	(72.1 - 18)	656	(73.4 - 184)	670	(66 - 529)
4	682	(77.5 - 38)	754	(70.1 - 668)	764	(62 - 415)
5	767	(51.7 - 77)	826	(54.3 - 602)	856	(53.9 - 203)
6	826	(51.2 - 97)	869	(49.3 - 264)	908	(57.8 - 159)
7	869	(55.1 - 87)	908	(64.5 - 178)	963	(67.6 - 32)
8	894	(63.1 - 51)	959	(56.5 - 129)	1006	(58.1 - 29)
9	936	(77.3 - 21)	991	(68.2 - 75)	1048	(43.1 - 30)
10	955	(63.7 - 19)	**1031**[a]	(68.3 - 33)	**1063**[a]	(45.6 - 19)
11	**991**[a]	(65.3 - 21)	**992**[a]	(103.4 - 13)	1069	(33.1 - 10)
12	**1004**[a]	(71.9 - 7)	**1029**[a]	(74.3 - 15)	**1096**[a]	(79.0 - 3)
13	**1049**[a,b]	(37.7 - 4)	**1031**[b]	(84.6 - 12)	**1109**[a]	(50.9 - 3)
14	**1066**[a,b]	(19.8 - 2)	**1063**[b]	(42.7 - 13)	**1108**[a]	(27.7 - 6)
15	**1092**[a]	(0 - 1)	**1103**[a]	(53 - 4)	**1151**[a]	(24.7 - 2)
16	**1064**[a]	(27.5 - 7)	**1057**[a]	(21.6 - 5)	1165	(0 - 1)
17	**1076**[c]	(50.3 - 9)	**1074**[a]	(72.7 - 5)	**1120**[a]	(0 - 1)
18	**1068**[a]	(22.6 - 10)	**1065**[a]	(176 - 2)	**1157**[a]	(0 - 1)

Table 6

Percentage of mature specimens by size interval for female gag collected during 2004-05. Specimens in the developing, ripe, spent, or resting stages were considered mature.
n = number of specimens.

Total length (mm)	% mature	**n**
451-500	0.0	1
501-550	0.0	11
551-600	2.6	76
601-650	6.3	159
651-700	43.8	121
701-750	89.3	122
751-800	99.5	185
801-850	100.0	192
851-900	100.0	165
901-950	100.0	91
951-1000	100.0	72
1001-1200	100.0	44
Total		1239

Table 7

Percentage of mature specimens by age interval for female gag collected during 2004-05. Specimens in the developing, ripe, spent, or resting stages were considered mature.
n = number of specimens.

Age (yr)	% mature	**n**
1	0.0	2
2	25.8	62
3	36.9	385
4	93.8	336
5	100.0	214
6	100.0	174
7	100.0	38
8	100.0	33
9	100.0	18
10	100.0	6
11-14	100.0	8
Total		1276

Size and age at 50% maturity of gag during three periods (1977-82, 1994-95, 2004-05). CI = confidence interval. Distrib. = cumulative distribution used in Probit model. Datasets from McGovern et al. (1998) and Harris and Collins (2000) were reanalyzed in the present study; differences in L_{50} between those reported below and the results of the published studies are due to the use of different size intervals.

Period	Size at 50% maturity				Age at 50% maturity			
	L_{50} (mm)	Distrib.	95% CI	n	A_{50} (yr)	Distrib.	95% CI	n
1977-82	630	Gompertz	602-650	471	3.7	Logistic	3.4-4.0	329
1994-95	620	Gompertz	610-629	3679	2.6	Logistic	2.4-2.8	1438
2004-05	680	Logistic	674-685	1239	3.2	Gompertz	3.0-3.3	1276

Table 9

Sex distribution by size interval for sexually mature gag collected during 2004-05. Specimens in the developing, ripe, spent, or resting stages were considered mature.
n = number of specimens, Trans = transitional.

Total length (mm)	Female	Male	Trans
451-500	--	--	--
501-550	--	--	--
551-600	2	--	--
601-650	10	--	--
651-700	53	--	--
701-750	109	--	--
751-800	184	--	--
801-850	192	1	1
851-900	165	--	1
901-950	91	--	1
951-1000	72	1	2
1001-1050	27	19	6
1051-1100	14	22	3
1101-1150	2	17	1
1151-1200	1	6	--
No length	113	10	2
Total	1035	76	17
Percentage	91.8	6.7	1.5

Table 10

Sex distribution by age interval for sexually mature gag collected during 2004-05. Specimens in the developing, ripe, spent, or resting stages were considered mature.
n = number of specimens, Trans = transitional.

Age (yr)	Female	Male	Trans
1	--	--	--
2	16	--	--
3	142	--	1
4	315	--	--
5	214	1	1
6	174	1	6
7	38	4	--
8	33	4	3
9	18	9	4
10	6	17	--
11	5	11	--
12	2	3	--
13	--	3	--
14	1	6	--
15-25	--	7	--
No age	71	10	--
Total	1035	76	17
Percentage	91.8	6.7	1.5

Size and age at 50% transition to male of gag during three periods (1977-1982, 1994-1995, 2004-2005). CI = confidence interval. Distrib = cumulative distribution used in Probit model. Datasets from McGovern et al. (1998) and Harris and Collins (2000) were reanalyzed in the present study.

Period	Size at 50% transition				Age at 50% transition			
	L_{50} (mm)	Distrib.	95% CI	n	A_{50} (yr)	Distrib.	95% CI	n
1977-82	995	Logistic	980-1013	501	11.7	Normal	10.5-14.0	322
1994-95	1024	Normal	1011-1041	3836	10.5	Normal	8.8-16.6	1506
2004-05	1049	Logistic	1030-1074	1003	9.7	Normal	9.3-10.3	1047

Table 12

Linear regression coefficients for the relationship between batch fecundity (BF; number of migratory nucleus and hydrated oocytes) and total length, fork length, total body weight, ovary-free body weight, and age in gag. Specimens were collected from Cape Lookout to Cape Canaveral with snapper reels by commercial fishers during January through May of 1996-97 and 2001-05. **P < 0.0001.

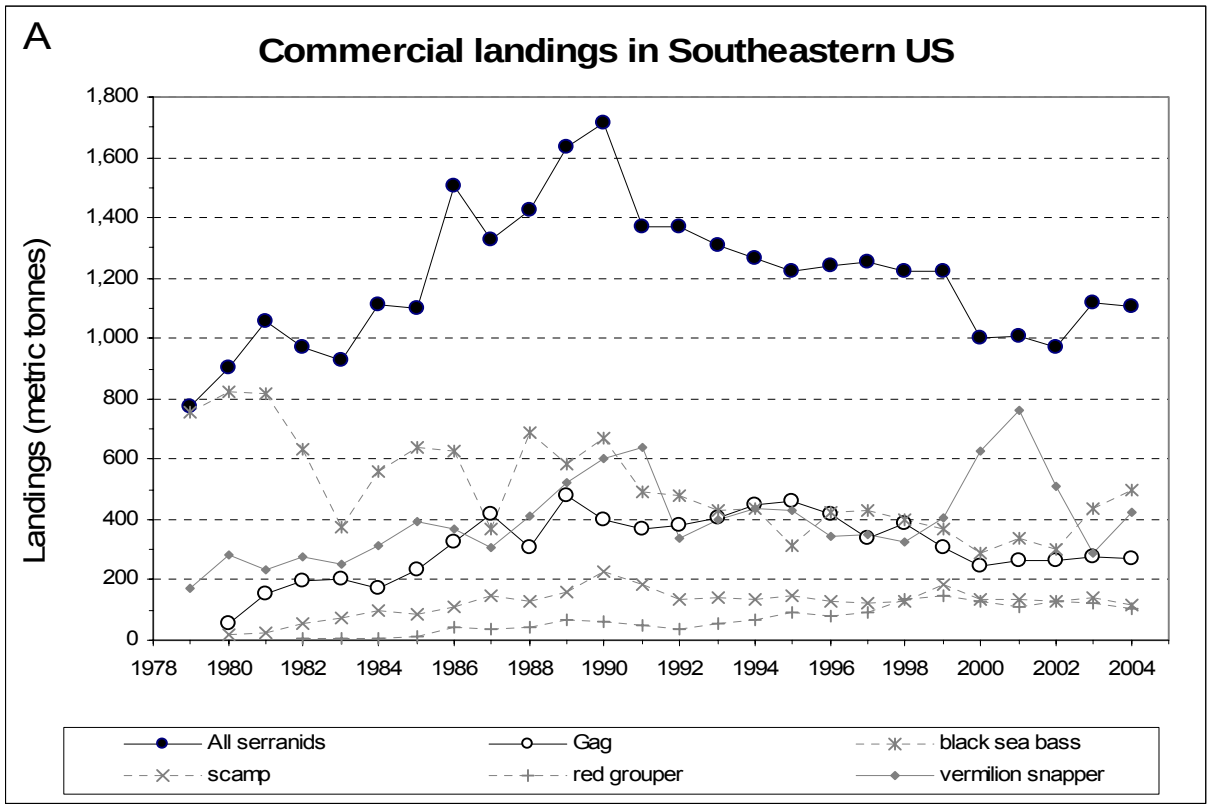
Linear equation BF = a + bX

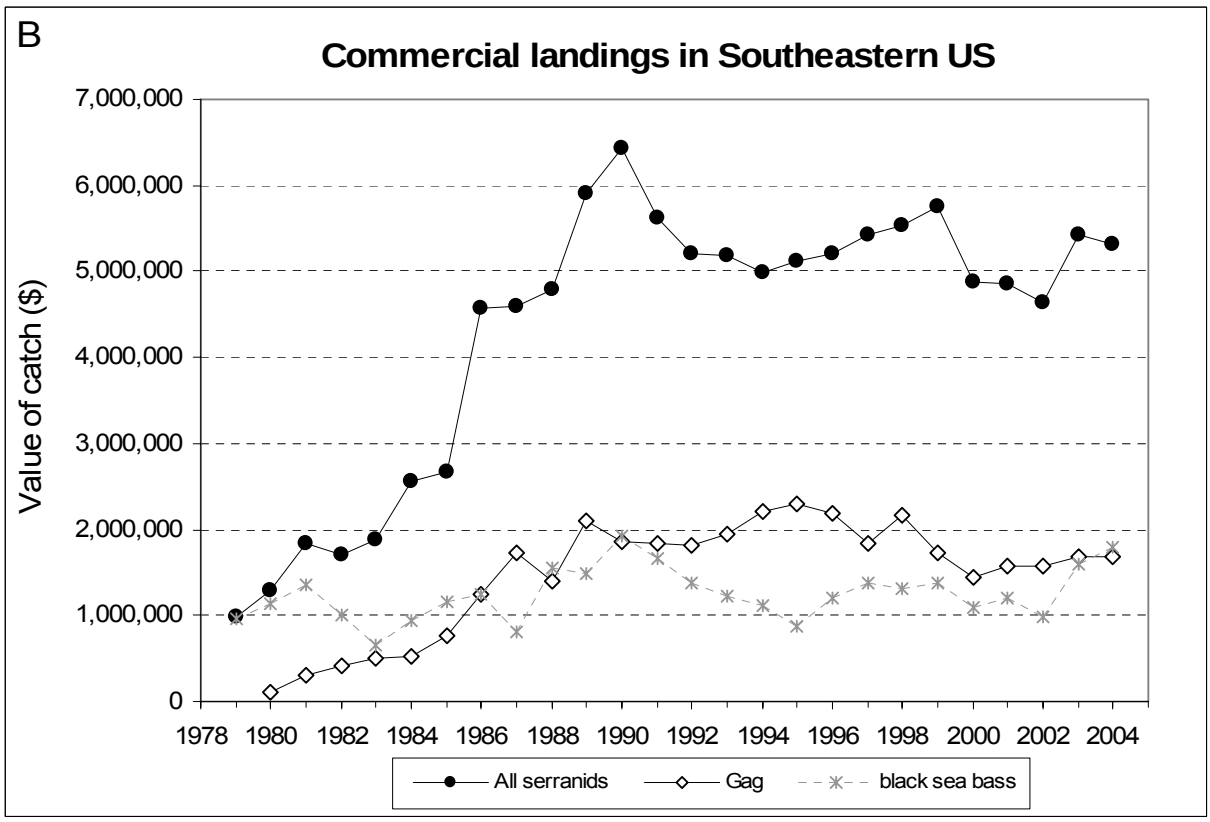
X	a	95%CI	B	95%CI	Adjusted r^2	F	N	Range of X
Total length (mm)	-975,902	± 459,624	1,671	± 529.9	0.294	39.25**	93	661-1159
Fork length (mm)	-1,047,145	± 472,315	1,821	± 561.2	0.290	41.43**	100	636-1096
Total weight	-130,790	± 134,536	68.14	± 14.70	0.500	84.97**	85	3,670-21,020
Ovary-free weight	-106,954	± 143,564	68.56	± 16.47	0.446	68.59**	85	3,515-20,139
Age	29,632	± 197,829	81,995	± 36,270	0.168	20.16**	96	2-8

Number of female gag with migratory-nucleus oocytes (MNO) or hydrated oocytes (HO) captured between 13 January and 6 May during 1996-97 and 2001-05, and total number of active (i.e., presence of vitellogenic oocytes) females captured during same period. The proportion of specimens with MNOs or HOs was used to estimate spawning frequency.

Month	No. with MNO or HO	No. of active females
Jan	4	58
Feb	10	58
Mar	80	176
Apr	97	184
May	4	14
Total	195	490
Proportion of total	0.398	

Commercial landings in metric tons (A), and dollar value (B) of all serranids, gag, and a few other important species in the snapper/grouper complex for comparison, as reported for the South Atlantic from 1979 through 2004. Landings from the most recent year may be inaccurate. Based on data from NMFS (http://www.st.nmfs.noaa.gov).





Commercial landings and recreational catches of gag along the southeastern Atlantic U.S. coast from 1979 through 2004. Data from 2004 may be inaccurate. Error bars around the means of the survey based recreational catches are ± 1 standard error. Data from NMFS (http://www.st.nmfs.noaa.gov).

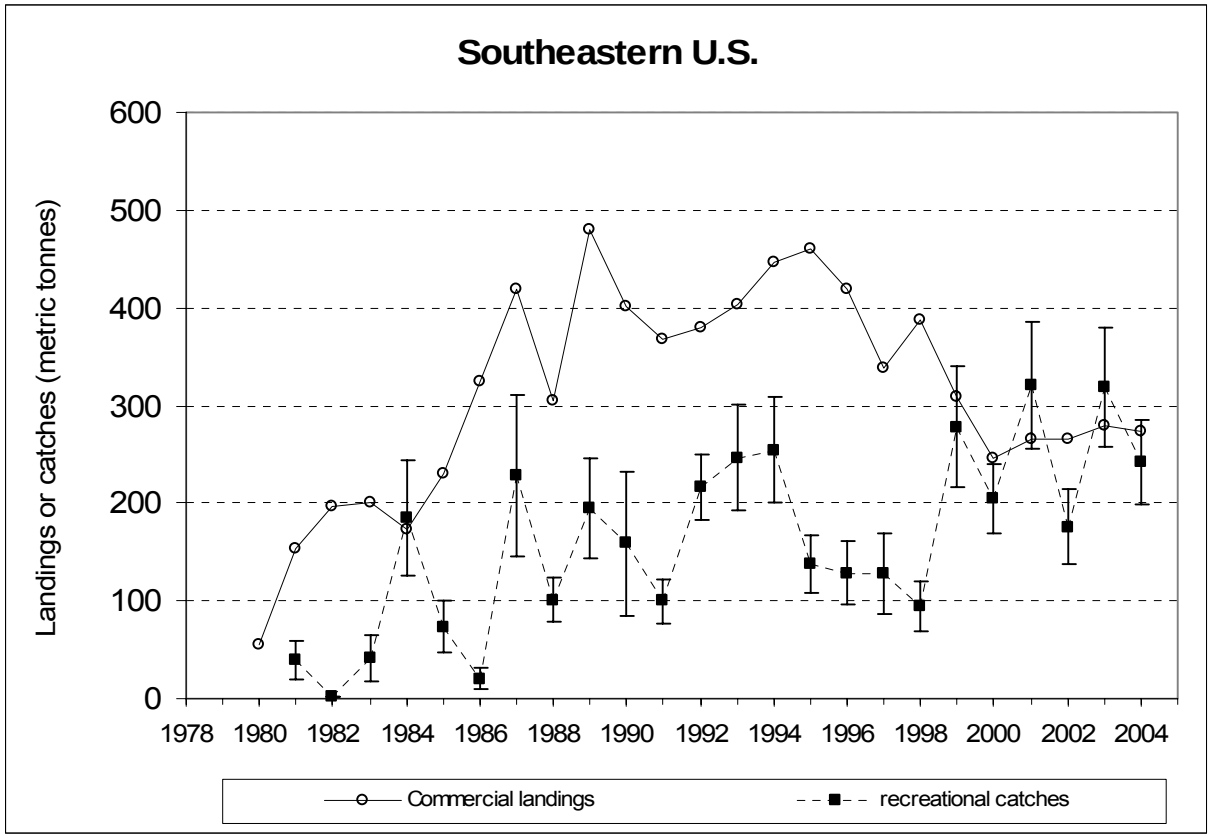
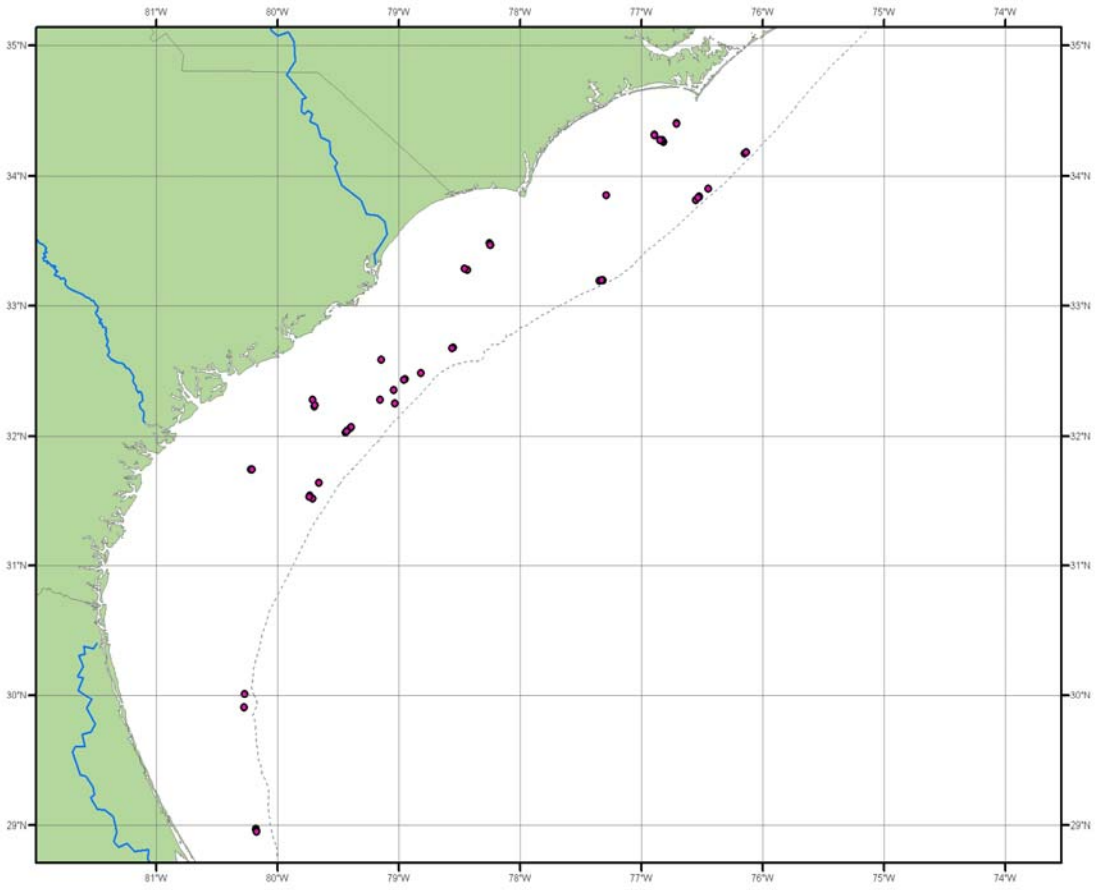
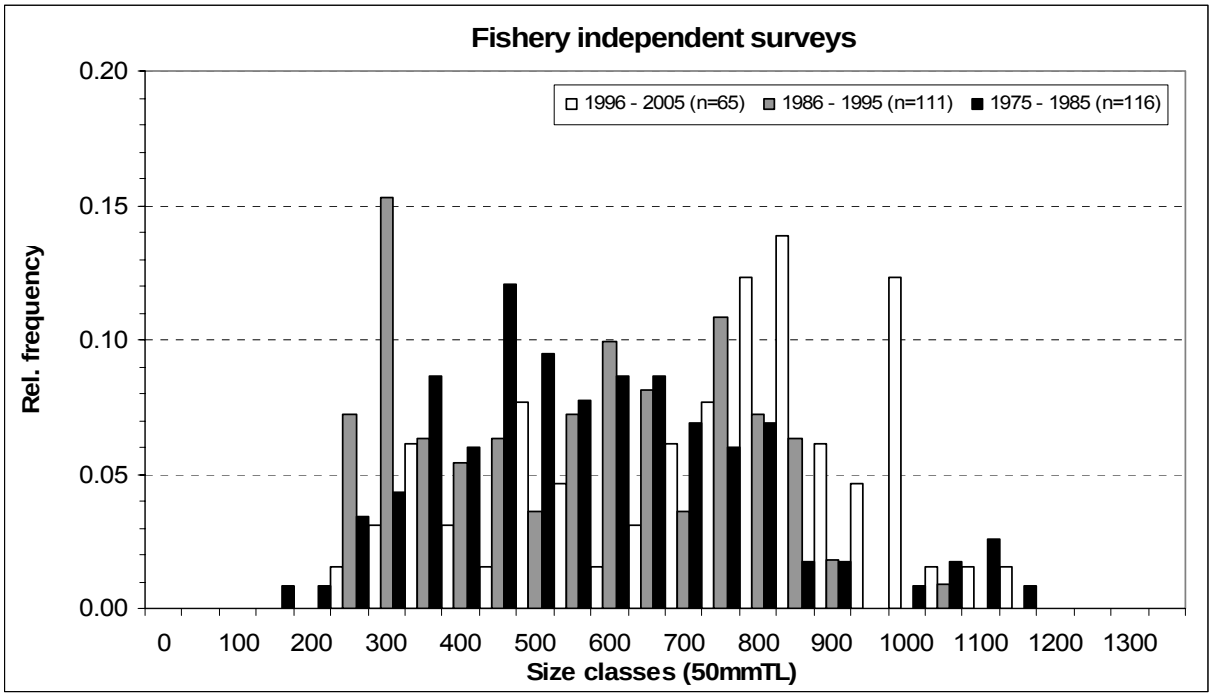


Figure 3

Locations of MARMAP collections for gag from 1996 to 2005 (closed circles) off the southeastern U.S. The dotted line indicates the 200 depth contour.

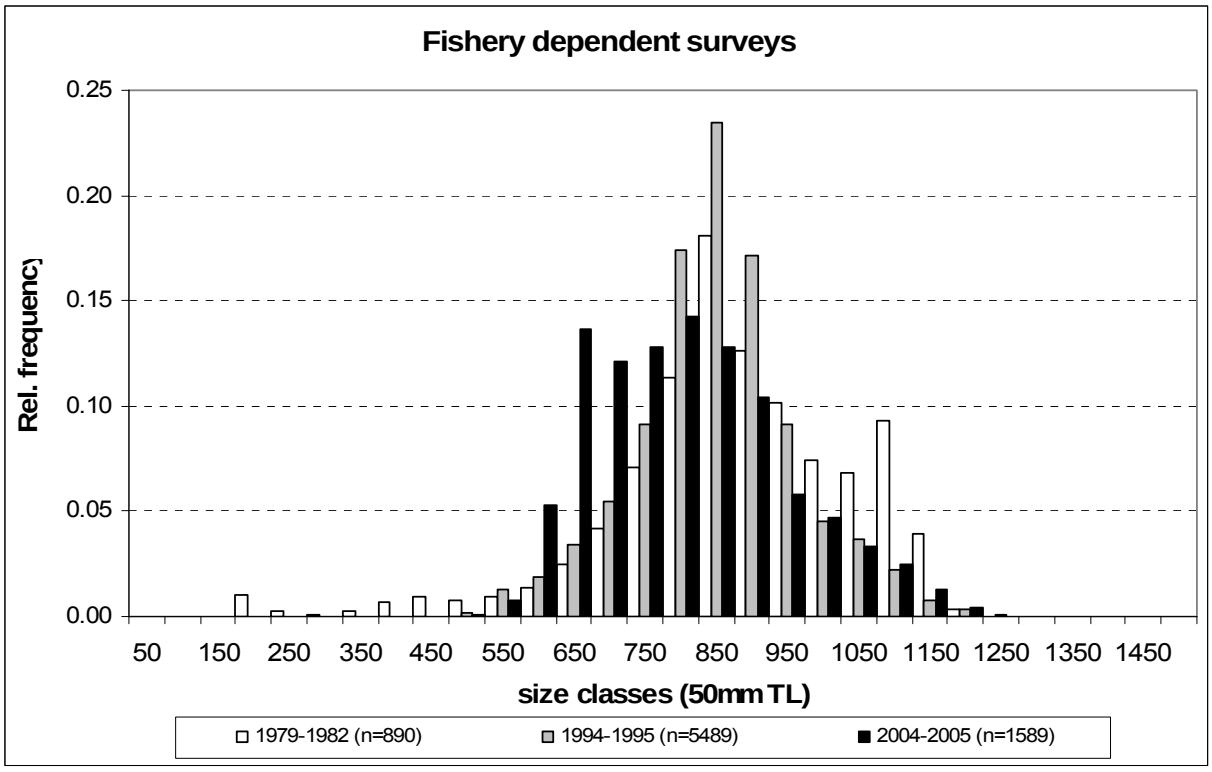


Length frequency of gag collected during MARMAP surveys off the southeastern U.S. in three periods. Lengths on the x-axis indicate the upper limits of the 50 mm total length (TL) intervals.



Length frequency of gag collected during fishery dependent surveys off the southeastern US in three periods. Figure 5A represents all fish while Figure 5B represents only the mature fish (see text for details). Lengths on the x-axis indicate the upper limits of the 50 mm size bins. TL=total length.

A



B

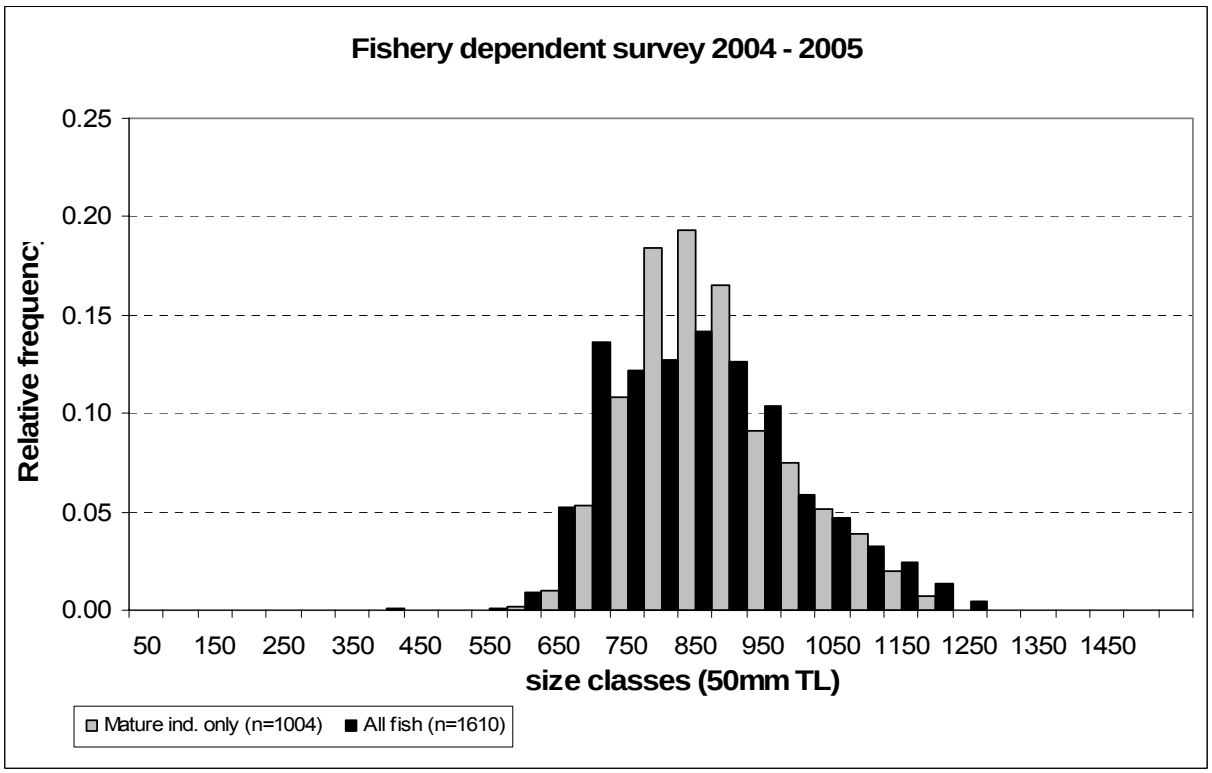
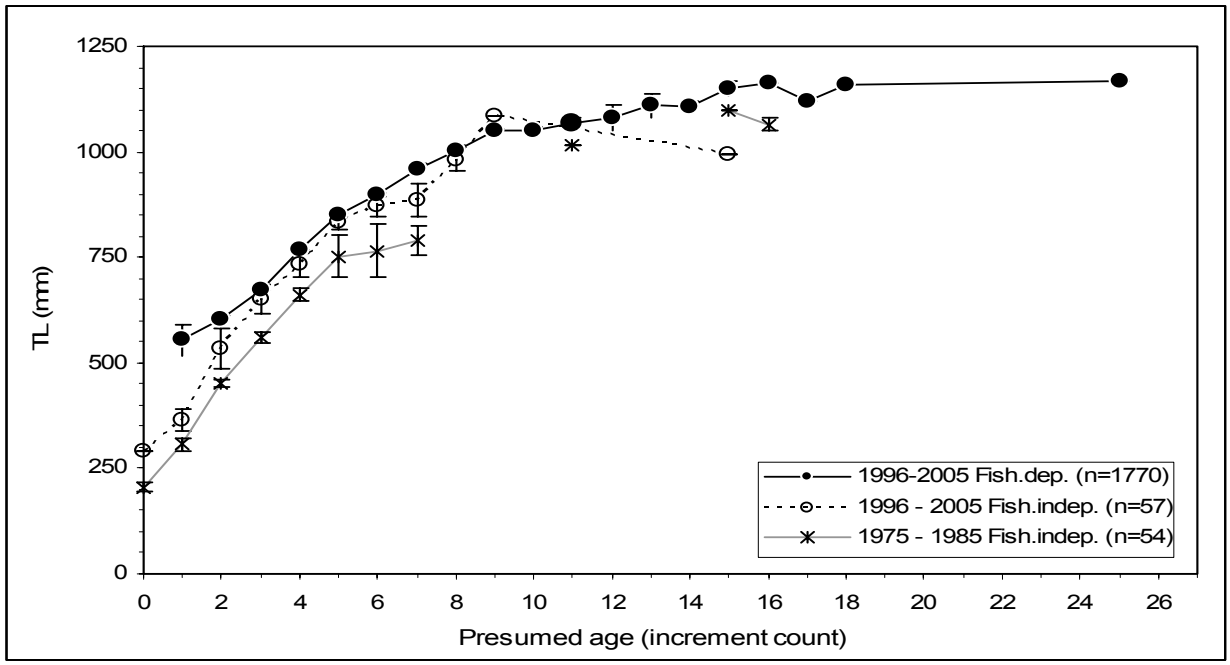


Figure 6 A – B

Length at age for gag collected off the southeastern U.S. in fishery dependent and MARMAP surveys between 1996 and 2005 (6A), and fishery dependent surveys in three periods: 1979-82, 1994-95, and 2004-05 (6B). See Table 4 for analysis details. Increment counts from otolith information were not converted to age (see text for details). TL=total length. Error bars indicate ± standard error around the mean. Note that in figure 6B only the upper and lower bars are provided for the 1979-82 and 2004-05 data respectively to maintain graph clarity.

A



B

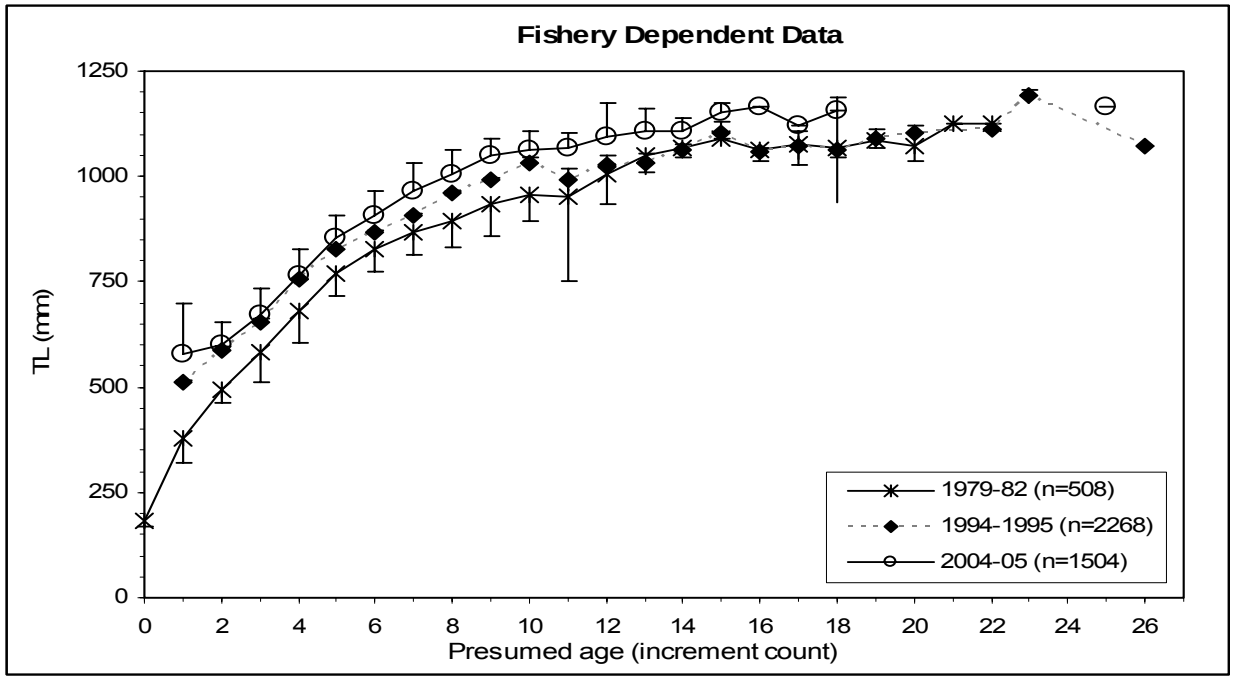
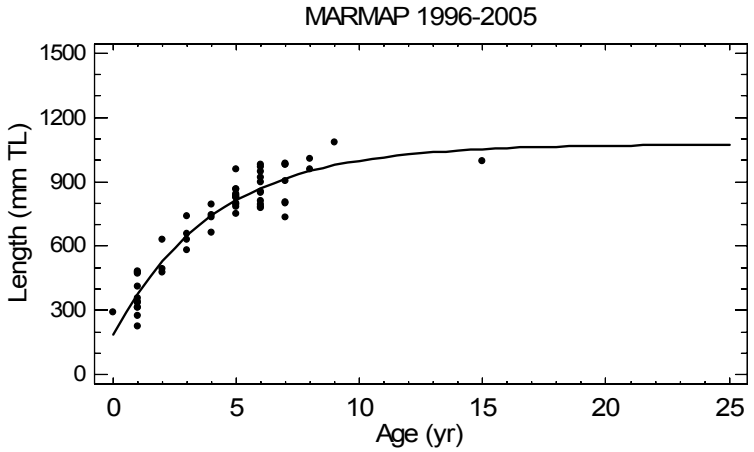


Figure 7 A - D

Von Bertalanffy (VB) growth curves for gag collected off the southeastern U.S. by the MARMAP surveys from 1996-2005 (7A), the fishery dependent survey from 2004-05 (7B), the combined MARMAP and fishery dependent data from 1996-2005 (7C), and the fishery dependent sampling periods of 1979-82, 1994-95, and 2004-05 (7D). Note that the age is actually the increment count of the examined otoliths.

A



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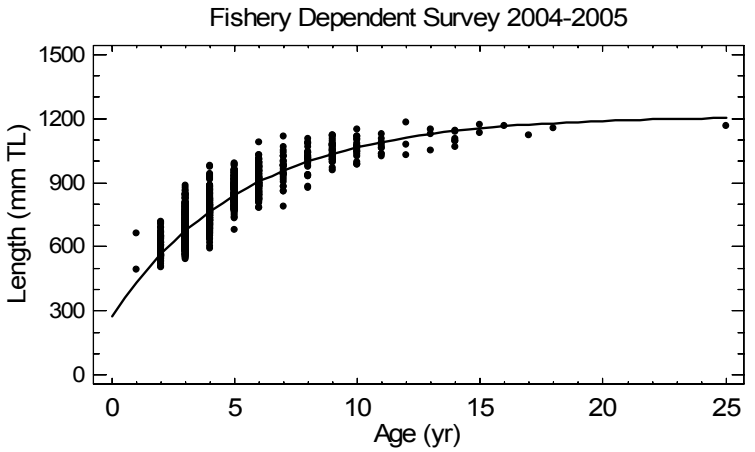
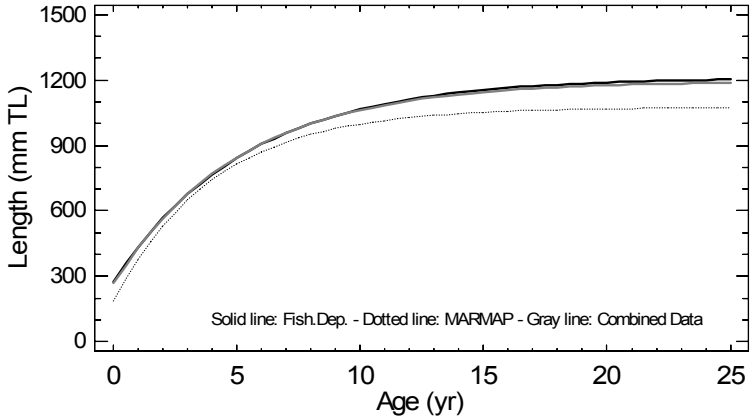


C



D

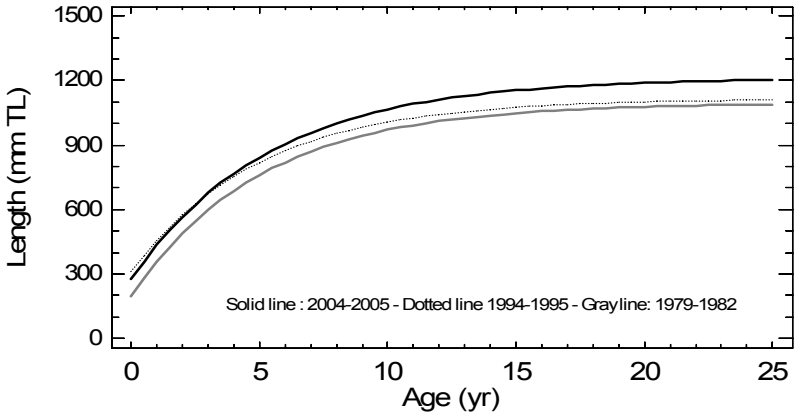


Figure 8. Comparison of length frequencies of female gag collected during 2004-05 that were categorized as immature (n = 317), definitely mature (n = 466), and resting (n = 382). Definitely mature specimens were mid to late developing (vitellogenesis through final oocyte maturation), ripe, or spent.

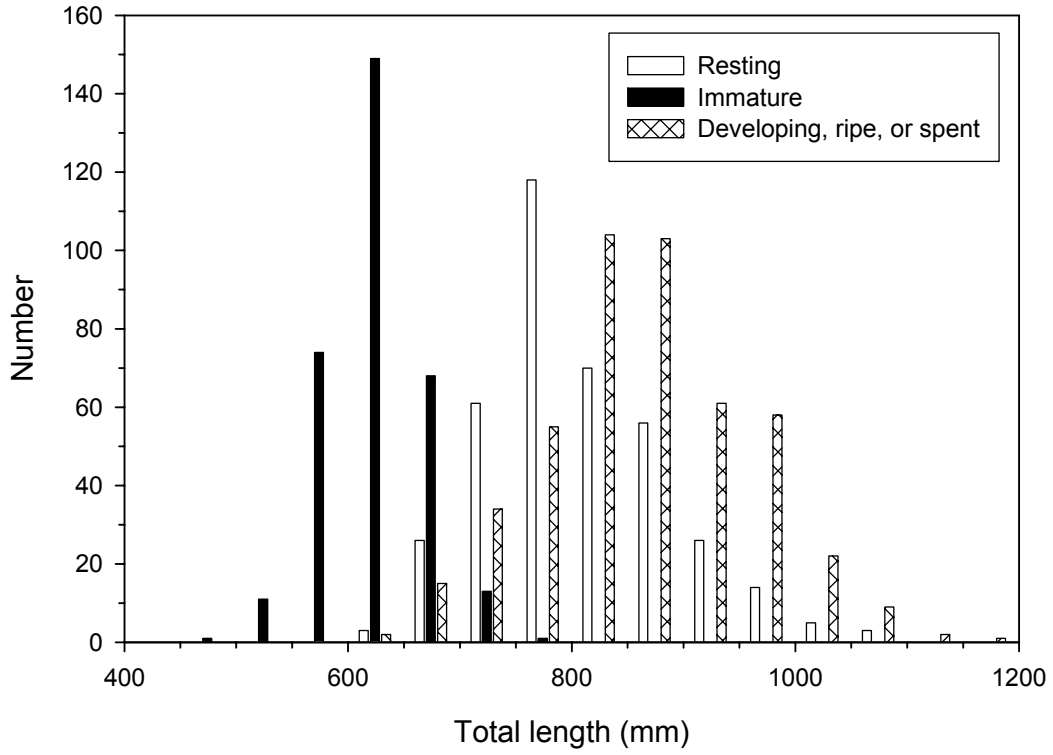


Figure 9. Comparison of length frequencies of female gag collected during 2004-05 that were categorized as uncertain maturity (n = 261), immature (n = 317), and resting (n = 382).

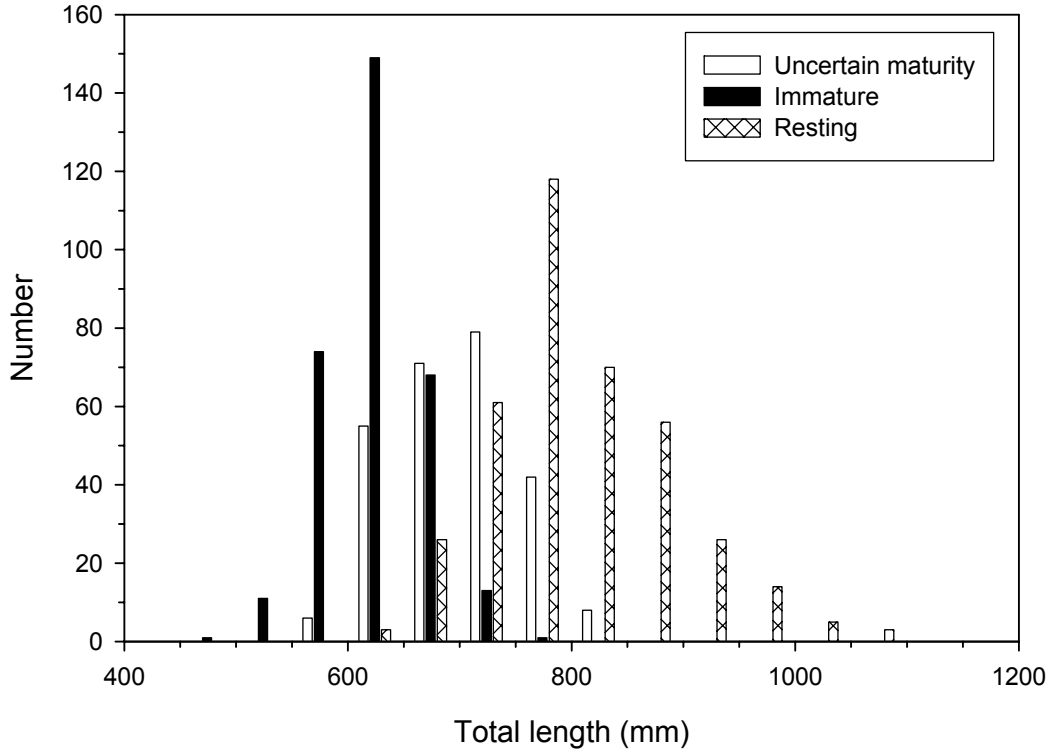


Figure 10

Estimates of total fecundity (number of stage-2 and stage-3 oocytes) in 86 gag relative to total length during four months. The specimens were captured with snapper reels in the commercial fishery off South Carolina and Georgia during 1996-97 and 2000-04.

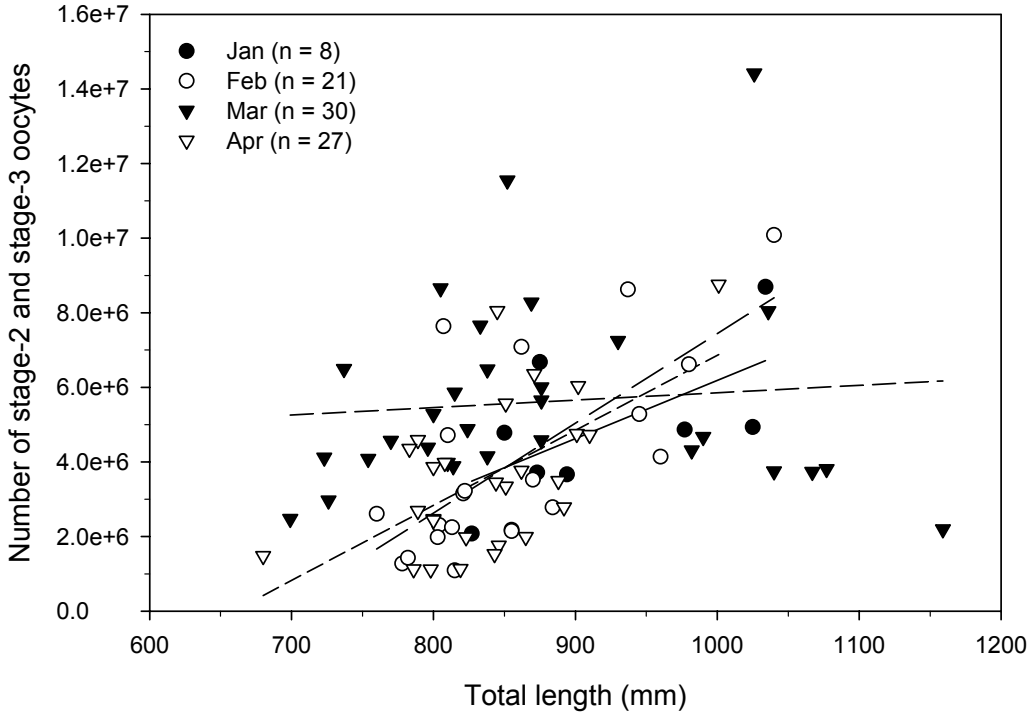
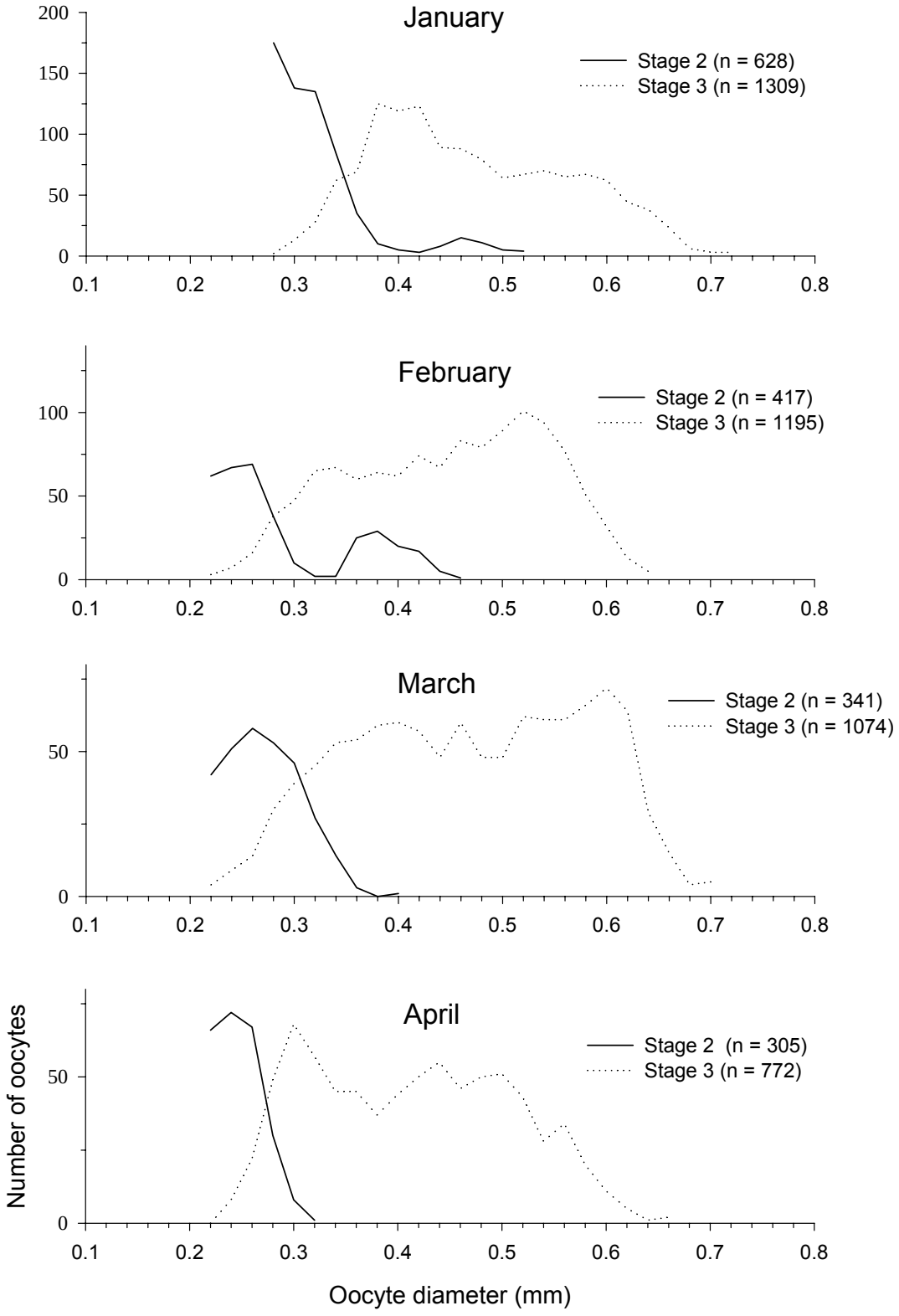


Figure 11

Number of oocytes by diameter for two stages of vitellogenic oocytes (see Hunter et al. 1992) in 20 gag (five specimens per month). Specimens were collected off South Carolina and Georgia with snapper reels by commercial fishers during 1996-97 and 2000-03.



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).

Marcel Reichert[1]
Gary Fitzhugh[2]
Jennifer Potts[3]

November, 2005

Addresses:

1 South Carolina Department of Natural Resources
Marine Resources Division MARMAP
Ft. Johnson Rd., Charleston, SC 29412

2 NOAA/National Marine Fisheries Service
Southeast Fisheries Science Center, Panama City Laboratory
3500 Delwood Beach Road, Panama City, Florida 32408

3 NOAA/ National Marine Fisheries Service
Center for Coastal Fisheries and Habitat Research
101 Pivers Island Road, Beaufort, NC 28516-9722

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, <u>we concluded that there was a high level of agreement between all readers</u>.
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.

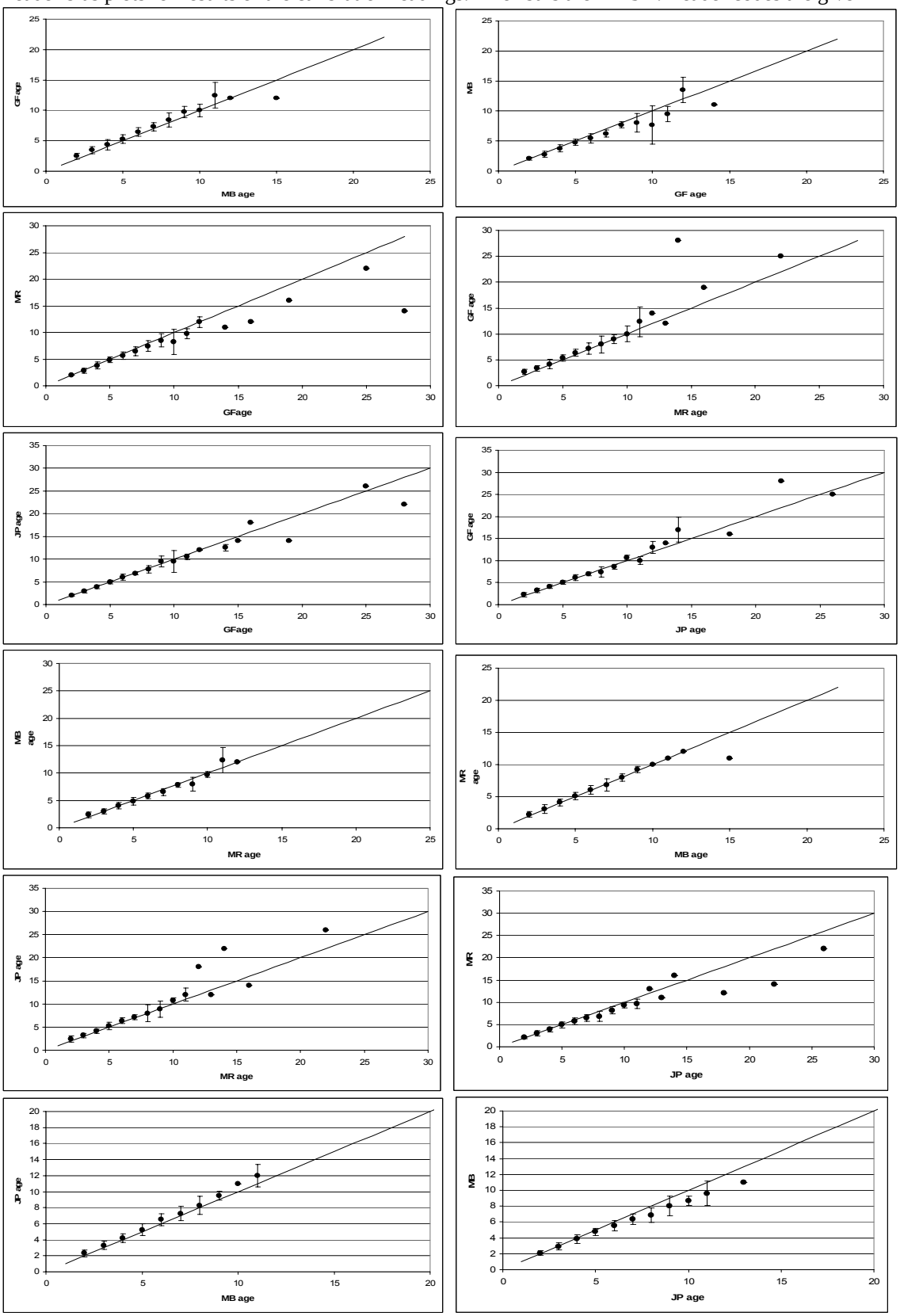


Figure 2
Reader bias plots for results of the calibration readings. Error bars are ± 1 SD. Reader codes are given in Table 5.