Draft Working Document

Update of gag (*Mycteroperca microlepis*) reproductive parameters:
Eastern Gulf of Mexico, SEDAR 10 Data Workshop

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Introduction

There have been several investigations of the reproductive biology of the gag, (*Mycteroperca microlepis*) underscoring the economic importance of this species. In the US South Atlantic and eastern Gulf of Mexico, studies have addressed reproductive seasonality, depth of spawning, sex ratio, maturity, sexual transition, aspects of the mating system, principal spawning habitats and regions, behavior, coloration, reproductive endocrinology, fecundity and spawning frequency (McErlean and Smith 1964, Collins et al. 1987, Gilmore and Jones 1992, Hood and Schlieder 1992, Coleman et al. 1996, Koenig et al. 1996, McGovern et al. 1998, Collins et al 1998, Harris and Collins 2000, Heppel and Sullivan 2000, Koenig et al. 2000, and Fitzhugh et al. 2005). Recently, information on timing of reproduction, maturity and sex transition have come from the southern Gulf of Mexico, providing an important Mexican contrast to studies conducted in US waters (Brule et al. 2003).

Our objective is to provide an update of the eastern Gulf of Mexico reproductive parameters most useful for the modeling efforts of the upcoming US Gulf gag stock assessment (SEDAR 10). This effort largely builds on previous efforts by Collins et al. (1998) in providing estimates of maturity, sexual transition, and fecundity. We make comparisons with other research efforts, particularly comparing eastern Gulf parameters to those estimated for Mexico (Campeche) and the US South Atlantic.

Methods

Sampling and processing

Efforts were made to obtain lengths (mm), weights (kg), gonads and otoliths from commercial and recreational fisheries, and fisheries-independent (scientific) surveys from the Gulf of Mexico (Lombardi-Carlson et al. 2006). Commercial samples were obtained from both bandit- and long-line boats. Recreational sector samples were obtained from

charterboats, headboats and private boats. Fish collected were weighed and measured, and the gonads were removed and placed in plastic bags and stored on ice. Gonads were shipped overnight and processed at the Panama City Laboratory. During processing, excess tissue was removed and each gonad examined microscopically. Most gonads sampled were weighed to the nearest 0.1 g and placed in plastic bags with 10% buffered formalin and later sectioned for histological observation. During the years prior to 1998, ovaries were selected for histological preparation to minimize processing time and costs. During this period, if there were a number of ovaries from a particular collection, at least n=5 were selected for sectioning as representative of the entire collection based on the microscopic examination. From 1998 on, all collections of ovaries were processed for histological observation. During all years, any obviously hydrated ovaries were identified and set aside for fecundity determinations.

Prior to 2002, a randomly selected region (anterior, medial, or posterior) on one or both lobes of the gonad was cross sectioned for collections. Starting in 2002, the posterior region of the gonad was consistently selected for sectioning (following Harris and Collins 2000). Gag ovaries are homogeneous with respect to oocyte size and counts of hydrated ova by location (Collins et al. 1998). The samples were placed in individual tissue cassettes along with formalin for histological slide preparation at Louisiana State University School of Veterinary Medicine, Department of Pathology. Otoliths were extracted and ages were determined according to Lombardi-Carlson et al. (2006).

Our work is an update and expansion of that provided by Collins et al. (1998) for maturity, sexual transition, fecundity and spawning frequency. There were some notable differences from the methods in Collins et al. (1998). Here, we estimate one overall spawning duration based on observations of earliest and latest spawning made over several years. Because sampling each year is haphazard and largely opportunistic, we felt we could not assume there was sufficient temporal coverage to provide year-by-year estimates of spawning duration. While acknowledging the haphazard nature of fishery-dependent sampling of reproductive tissues, we assumed that samples selected and processed histologically were randomly sampled. We know of certain exceptions, such

as directed sampling of copperbelly pigmented fish in 1994 (Collins et al 1998). In other cases, we did record whether a fish was selected (because of size or pigmentation) when that information was provided to us by the port agent. Parameters that are simply related to age or size such as male transition, maturity and fecundity should be robust to haphazard sampling.

Maturity

Females displaying vitellogenic or more advanced oocytes (yolked oocytes) were defined as "definitely mature" (consistent with terminology used in MARMAP study; D. Wyanski personal communication). Females with cortical alveoli (CA) or primary growth oocytes (PG) as the leading stage, but displaying atretic-yolked oocytes, loosely packed lamellar folds, and evident melanomacrophages were also classified as mature but were further distinguished as "resting mature" (similar to McGovern et al. 1998). Females were also classified as "spawning" depending upon the presence of hydrated oocytes, indicative of imminent spawning, or postovulatory follicles (POF), indicative of recent spawning. Our distinction of hydration also includes migratory nucleus stage oocytes undergoing yolk coalescence. Females with PG oocytes as the leading stage, with no atresia of yolked oocytes and minimal to zero melanomacrophages were deemed immature. However, it was not uncommon to note females possessing primary growth oocytes, together with some atresia of unknown oocyte types and/or exhibiting a few and difficult to detect melanomacrophages. These females were classified as "uncertain" maturity. Specimens were assigned to 5 cm total length classes and the proportion "definitely mature" was related to length classes using logistic regression weighted by the numbers in each length class. The logistic model, based upon the Gompertz function, where P_x = proportion mature in each length or age class, was fitted to the data using maximum likelihood (logistic regression, XLSTAT version 7.5 analytical software). Model fits were compared using McFadden's r^2, a modified determination coefficient used in Logistic regression (XLSTAT version 7.5 manual and software).

Female activity

Because mature adult fishes may not spawn every year or because spawning can be disrupted (e.g., Rideout et al. 2005), we also screened histology sections for evidence of unusual atresia patterns and for inactive females that may have spawned in previous years but were foregoing spawning in the current year. These inactive or skipped spawners would show signs of previous spawning including presence of melanomacrophages (brown bodies) or remnant hydrated oocytes external to the lamellae, but would not have advanced to vitellogenesis by the months of peak spawning (February and March).

Spawning frequency

Spawning frequency was estimated based on the average spawning proportion of mature females showing hydrated ova (Day-0 proportion, Fitzhugh et al. 1993, Nieland et al. 2002) out of the total definitely mature females (determined histologically). This approach is improved somewhat over that conducted by Collins et al. (1998) in that we had more histological samples completed to assign females to maturity stages (rather than counting females above some size threshold). The inverse of the spawning proportion yields the frequency: the average expected interval in days between spawning events. The overall spawning season duration in days divided by the average interval yields the expected number of spawns per female per annual reproductive season.

Fecundity

Batch fecundity was determined using the hydrated oocyte method. Ovarian tissue samples were cross sectioned, weighed to the nearest 0.001 g and placed in a vial along with 33% glycerol to separate oocytes for the purpose of counting (Collins et al. 1998). Batch fecundity was calculated by multiplying the final hydrated ooctye estimate by the whole ovary weight, and the product was divided by the weight of the sample (Collins et al. 1998). Batch fecundity was regressed on total length (TL), gonad-free body weight weight (Wt), and age for all hydrated females (Collins et al. 1998). Any sections showing recent post-ovulatory follicles, suggesting the female had partially spent her current batch, were eliminated from the fecundity estimates.

Since reported in Collins et al. (1998), our procedure for batch fecundity determination has been slightly improved by using two random ~ 0.075 g samples per ovary. The location of each of these two samples in each ovary was selected by two rolls of a die: first to select the longitudinal region of the sample-location (there were 6 regions in each ovary: anterior, middle or posterior on each lobe) and second to select either the periphery or center portion of the cross-section. The counts from these longitudinal and cross-sectioned selected samples were then averaged. This method of estimating batch fecundity samples is very similar to that used in recent publications (e.g., Harris et al. 2002) and is a better technique for dealing with fishes having large gonads. Any archived tissue samples remaining from Collins et al. (1998) were re-counted using this technique. If a female's fecundity was previously estimated but no tissue samples remained to be re-counted (this occurred for 16 females), an updated fecundity estimate was made based upon a regression of samples that were counted using the old and new method (new fecundity = 1.193 * old fecundity – 12276, $r^2 = 0.88$).

Sex change

Gag are known to be protogynous hermaphrodites (female first, changing to male). We identified transitional and male gag based on histological observations from samples provided for gonad analysis. Similar to the analysis for maturity, specimens (female, transitional, and male) were assigned to 5 cm total length classes and the proportion transitional and male was related to length classes (midpoints) using logistic regression weighted by the numbers in each length class. The logistic model, based upon the Gompertz or Logit function where P_x = proportion female in each length and age class, was fitted to the data using maximum likelihood (logistic regression, XLSTAT version 7.5 analytical software).

Otoliths were collected by port agents often independent of gonad sampling and in greater numbers than were gonad samples (see Lombardi-Carlson et al. 2006 for otolith sampling description). Often, the visual appearance of dark belly pigmentation ("copperbelly", see photo in Appendix 1) on the fish was noted by the port agent on the

otolith envelope. Gag with copperbelly pigmentation have been shown to be mostly males (>90%, Collins et al. 1998). These observations represented a potentially larger sample size than offered by gonad sampling, however port agents were not consistent in reporting visual copperbellies; there was certainly no requirement to do so. Sometimes copperbellies were reported but not always. Often a collection of fish, say 7 individuals, would contain one fish visually identified as a copperbelly. Based on discussions with several port agents, it would be a safe assumption that the 6 fish not designated as copperbellies in that particular collection did not have the pigmentation. Therefore, we constructed a sample set containing gag from all collections containing at least one visual copperbelly as this set would include, with fair certainty, fish noted to be copperbellies and fish noted not to be copperbellies. Similar to the approach used to estimate size at sexual transition from histological observations, we repeated the analysis by fitting the proportion of unpigmented fish (presumed to be females) in each length and age class to the logistic model.

Results and Discussion

<u>Sample characteristics</u>

A total of 3,722 gag with gonads were sampled (weighed and macroscopically examined) from the U. S Gulf of Mexico, primarily from the Keys to the Florida panhandle region (Monroe to Bay County 99%). Of these, 2,162 gonads were thin sectioned and stained for histological examination. Sampling was conducted largely between 1991 and 2002 with most collections (69%) made during years 1991 to 1996 and 31% from years 1997 to 2002. Samples were taken throughout the year but a majority of gonads (54%) were sampled between January and the end of April encompassing the spawning season. Most histologically assessed samples came from the commercial hook-and-line sector (53%), followed by the recreational hook and line sector (29%; including charter boat, headboat and private sources), the commercial long-line sector (13%), and an "other" category (5%) including sources such as tournaments, spear-fishing and scientific surveys. Fishery-dependent sampling of gonads deviated somewhat from the age structure sampling (Lombardi-Carlson et al. 2006) in that reef

fish are not commonly landed with gonads intact, and we were dependent upon cooperative fishermen and extra efforts by several port agents to make the collections.

Since copperbelly pigmented gag are known to be primarily male and transitional fish (>90%, Collins et al. 1998), we felt information about size and age of gag at sexual transition could be obtained from the notations about copperbelly pigmentation often made by port agents when they collect otoliths. This approach would then provide an independent estimate from that obtained using histological samples from gonads. Since 1991 there were 436 collections containing at least one visually detected copperbelly; yielding a total of 796 copperbellies out of 1793 gag (total fish in collections containing at least one copperbelly gag). Most of these visual copperbellies (82%) were reported from 2001 to 2004, a period in which otolith sampling increased (Lombardi-Carlson et al. 2006).

Reproductive stages and season duration

Based upon female gag noted to be in spawning condition (exhibiting hydrated oocytes [H] or showing postovulatory follicles [POF]; all years combined), the earliest date of spawning is day 26 (late January); latest is day 116 (about April 27) giving an estimate of 91 d spawning duration (Figure 1).

Spawning females (H and/or POF) were largely greater than 600 mm TL. When all years were examined, only two individuals were below this size (513 and 577 mm TL, Figure 1) which is similar to the previous finding of minimum size of a spawning female (Collins et al. 1998). However, the smallest female exhibiting cortical alveolar stage (CA) oocytes equaled 449 mm TL. Additionally, there were a number of females under 500 mm TL exhibiting CA oocytes as the leading stage. Some investigators consider females exhibiting the CA oocyte stage to be mature as this stage typically appears at the beginning of seasonal development and advances to vitellogenesis in many species examined (e.g., Harris et al. 2002). Since the smallest females exhibiting CA stage oocytes were much smaller than females observed in spawning condition (H and POF), it seems that the presence of the CA oocyte stage in and of itself may not be a certain

indication that a female gag will progress to spawn in the current season. The smallest female exhibiting vitellogenesis was 504 mm TL and females in this stage overlapped more closely in size with those females showing positive indications of spawning (H and POF). Therefore, we distinguished females with vitellogenic or more advanced oocytes as "definitely" mature.

In comparison of length frequencies by gonad development category, immature fish were clearly separated by size mode from resting mature (exhibiting indicators of prior spawning) and definitely mature females (Figure 2). There were some females between 750 and 850 mm TL, that were scored as immature (no clear indicators of prior spawning were evident), and it may be likely that some of these fish were misclassified and should be categorized as resting mature. We scored many females as having uncertain maturity, and these fish largely overlapped in size with resting mature females. A higher proportion of "uncertain mature" fish in the 400-700 mm TL size groups indicates that many of these fish should probably be categorized as immature (Figure 2).

Discriminating between resting mature and immature females can be challenging and was a main subject of a recent fish reproductive histology workshop in the southeast (Anonymous 2005). Based on the results of this workshop and recent exchanges with other laboratories, our criteria for separating resting mature from immature females is being updated. Using definitely mature females only (exhibiting vitellogenic or more advanced oocyte stage) to estimate size and age at maturity resulted in fewer females available by size and age groupings for regression. However, categorizing and counting vitellogenic and more advanced stage females as mature matches what most investigators have done in the past, and we feel this minimizes potential misclassifications. In the future, improved resolution of immature and resting mature fish may improve sample sizes among rare size and age groups and facilitate regression analysis.

Maturity

Based on definitely mature and immature gag, a logistic regression using the Gompertz function resulted in good model fits for size and age (McFadden r^2 =0.6 for

both regressions). The 50% length and age at maturity was estimated to be 585 mm TL and 3.7 years respectively (Figures 4 and 5) and our smallest female exhibiting vitellogenesis was 504 mm TL. Almost all fish were mature upon reaching 700 mm TL and 5 years of age.

Hood and Schlieder (1992) reported maturity of gag by age from the eastern Gulf during a much earlier sampling period (1977-1980). Their results are remarkably similar; some gag were found to be mature as early as age 2, but primary maturity occurred between ages 3-4, with about 70% of females mature by age 4 and 100% by age 6. Harris and Collins (2000) found 50% age at maturity for gag in the south Atlantic decreased from 3.8 years in 1976-1982 to 2.8 years in 1994-1995.

Our findings are also similar to, or perhaps slightly smaller than, size at maturity reported previously in US waters. Coleman et al. (1996) reported 50% length at maturity was 61.5 cm TL from the eastern Gulf; the minimum size at maturity at 49 cm TL, and all gag > 66 cm TL were mature. McGovern et al. (1998) noted length at 50% maturity in the US South Atlantic was 641 mm TL in 1978-1982 and 622 mm in 1994-1995; smallest mature females were observed at 508 mm in 1976-1982 and 517 mm in 1994-1995. All gag >683 mm in 1976-1982 samples and all gag >750 mm in 1994-1995 samples were sexually mature.

In the southern Gulf (Mexican waters), Brule et al. (2003) found the median size (L_{50}) at maturity for gag to be 72.1 cm, with the smallest mature female at 70.5 cm, and noted all females above 87.6 cm to be mature. This Mexican study reflects a substantially larger size at maturity than noted in US waters. We note that Brule et al. (2003) uses similar definitions and interpretations for maturity as US studies and this was confirmed at a recent histological workshop (Anonymous 2005). Thus the US-Mexican difference is not likely due to differences in methods. However, Brule et al. (2003) indicate that a relatively small number of samples were available in the sizes where onset of maturity occurs (n=10 between 50 and 70 cm, Fig. 5 in Brule et al. 2003), and this may have affected their logistic regression.

Spawning activity

        In examining the histology slides, we looked for any unusual atresia patterns occurring within fish gonads and whether atresia might indicate disrupted spawning as opposed to a more "normal" pattern. A recent review reported that skipped or disrupted spawning may be rather common as investigators learn better how to detect occurrences (Rideout et al. 2005).

        We did note conditions of significant atresia of CA or V oocytes, reflecting a hiatus in development or reproductive activity during the current year. In most of these atresia cases, the pattern involved significant atresia of vitellogenic oocytes in late March and April, likely to be the "normal" end of season shutdown (i.e., spent females). In only in 3 females we detected notable atresia of CA oocytes earlier in the season (February 11, 1994), all in the same collection. Thus, there was very little evidence for females who have begun the cycle of ovarian development (recrudescence) to halt or reverse the process during the normal course of the season.

        More evident than possible disruptions in reproductive development were occurrences of mature females (showing evidence of previous spawning) who appeared to fail to advance past the primary growth or cortical alveolar oocyte stage during the current spawning season. Potentially, these females were "skipped spawners". By examining females collected only during the peak period of spawning (February and March), we felt any females which showed evidence of prior spawning but only possessed primary growth oocytes as the leading oocyte stage, were not likely to advance to vitellogenesis and spawning. Our counts by year and by age indicated that they comprised about 9-10% of mature females (Table 1).   This appears to vary by year but sample sizes are quite small in some years. It does seem evident that younger females (age 3-7) are more likely to be inactive than older ages (Table 1). Misclassifications of maturity (being wrong about interpretations of prior spawning) might explain this. However, these February - March (PG stage) inactive females ranged up to 1190 mm TL ($45\% \geq 750$ mm TL) which is quite large and distinct from females scored as immature

(Figure 2). Coleman et al. (1996) also reports some gag females collected during the spawning season that they scored as mature but showed no development stage oocytes or signs of imminent spawning. Additionally, other investigators of gag reproductive biology have concurred that these oocyte patterns may reflect skipped spawning (D. Wyanski, T. Brule, personal communication, Anonymous 2005). This remains an area needing further investigation.

Spawning frequency

We found little evidence for a relationship between spawning frequency and age (p for weighted regression = 0.77, r^2=0.015, Figure 6). The mean spawning frequency estimate across ages in the regression is 3.7 d. Estimating across years (weighted mean) yields an expected spawning frequency of 4.0 d (sd =1.8). Based on a duration of 91 d this frequency yields an expected number of annual spawns per female equal to 23 or 25 (years averaged and age averaged respectively).

Fecundity

Batch fecundity increased with age and length of females, ranging from 60 thousand to 1.7 million ova per batch with a mean of 422 thousand ova (sd = 295 thousand). Variation in batch fecundity was generally high among age and size classes but the variation explained by linear fits of batch fecundity regressed on age and size were similar (r^2 = 0.30 and 0.34 respectively; Figures 7 and 8). As is common among fishes, the batch fecundity relationship was best predicted by regression with (ovary free) body weight (r^2= 0.53; Figure 10). This is similar to results given in Collins et al. (1998) but expands the sample size of hydrated females. Given there is little evidence for an age effect on spawning frequency, annual fecundity at age would merely be the product of the expected number of spawns per female per season multiplied by batch fecundity at age.

Sex change

The histological and visual analyses of female size at transition to male yielded very similar results. Based on histological criteria, 50% size at transition was 1100 mm TL (Figure 10), and based upon visual pigmentation, size at 50% transition was 1085 mm

TL (Figure 11). In both analyses, transition appeared to begin after 800 mm TL and nearly all gag had undergone transition upon reaching 1300 mm TL (Figures 10 and 11). Moreover, this is about the maximum size observed; our largest gag, which is noted to be a copperbelly in our data base, was 1384 mm TL. By comparison, our largest observed histological male was 1360 mm TL, largest transitional fish (of n=5) was 1101 mm TL, and largest histological female was 1330 mm TL.

It was apparent that the reduced number of males and transitionals at age (histologically examined) hampered the curve fit, and only 144 males and transitionals were available out of 1692 total fish observed by age. However, the visual pigment pattern data readily revealed an age relationship (Figure 12). Age at 50% transition was 10.8 years. Transition to copperbelly pigmentation began at age 7 and nearly all fish were pigmented after about 15 years of age.

Our size/age at transition data almost exactly matches that reported in Hood and Schlieder (1992) from their earlier time period (1977-1980) in the eastern Gulf. They reported nearly all fish less than 800 mm to be females, and 50% transition to have occurred by 1050 mm TL and age11. Similar results were found from the southern Gulf: 50% size at transition was 103 cm TL and males and transitionals in the Mexican data set ranged from 85 to 111 cm TL (Brule et al. 2003). McGovern et al. (1998) also reported that gag less than 800 mm were females except for a single transitional fish.

While most copperbellies have been found to be male (>90%, Collins et al. 1998), there has been little indication in the literature whether un-pigmented gag might be males. Koenig et al. (1996) indicated that they observed some gag that were histological males that weren't earlier noted to be copperbellies during sampling. Since our estimates of size at sex change and size at pigment change correspond so closely, it seems evident that pigment pattern does predict sex for the most part. Just to check, we sorted the visual pigmentation data set and found that 268 gag were unpigmented (with fair certainty; see methods) and happened to have a histological slide made for their gonad. Of these, 6 or about 2% were male and 98% were female.

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Appendix 1. "Copperbelly" gag (bottom) contrasted with unpigmented gag.

Year	CA or more advanced stage oocytes	PG stage oocytes	Age	CA or more advanced stage oocytes	PG stage oocytes
1991	116	10	2	1	
1992	8		3	11	1
1993	62	1	4	43	8
1994	99	8	5	114	22
1995	81	6	6	103	17
1996	30	1	7	54	2
1997	2		8	41	
1998	46	4	9	17	
1999	86	20	10	23	
2000	3	1	11	3	
2001	34	5	12	3	
2002	4	4	13	2	
			14	5	
			15	1	
			16	2	
Totals	571	60		423	50

Table 1. Mature females from the peak spawning months of February and March were categorized and counted according to the leading oocyte stage present in their ovaries (cortical alveolar, CA, or more advanced oocytes, and primary growth, PG, stage oocytes). The PG stage females counted here showed evidence of prior spawning (considered mature) and thus represent potential skipped spawners.

Year	No. Hydrated	Total definitely mature	Proportion spawning	Spawning frequency
1991	56	179	0.31	3.20
1992	4	8	0.50	2.00
1993	31	59	0.53	1.90
1994	10	64	0.16	6.40
1995	26	88	0.30	3.38
1996	11	55	0.20	5.00
1998	7	55	0.13	7.86
1999	29	97	0.30	3.34
Totals	174	605		

Table 2. Spawning frequency (average interval in days between spawning events per female) for years in which females with hydrated (H) oocytes were observed and estimated as a proportion of the total definitely mature females sampled during the spawning season. Weighted mean = 4.0 d (sd = 1.8).

Figure 1. Female gag, all years combined, noted to be in spawning condition (exhibiting either hydrated ova or postovulatory follicles). Earliest date of spawning condition is January 26; latest is day 116 (about April 27) giving an estimate of 91 d spawning duration. N=362.
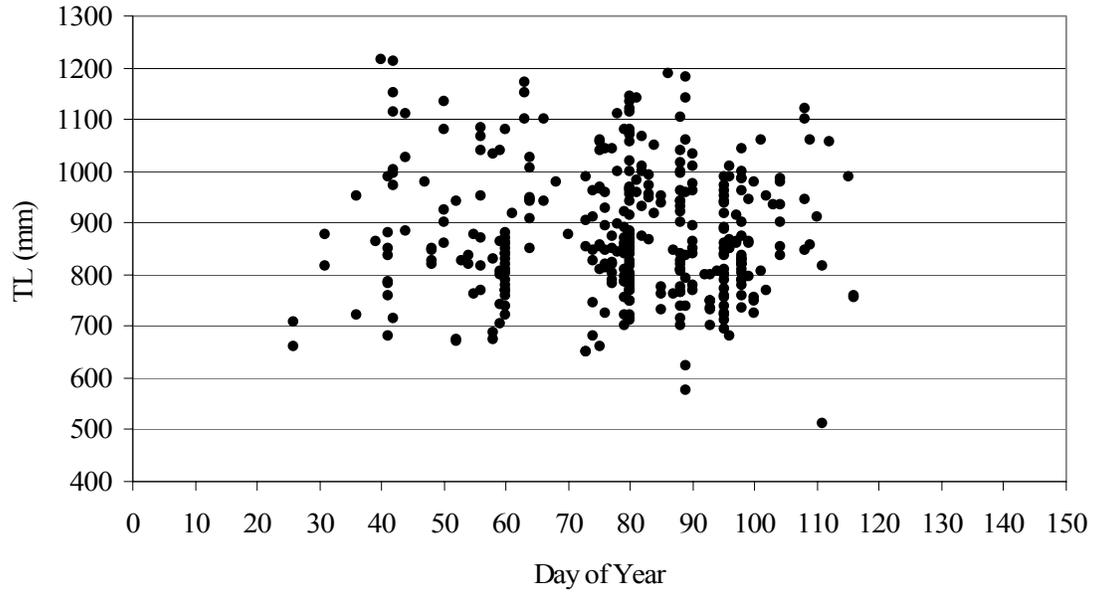
Figure 2. Size frequency histograms of gag histologically assessed and categorized as definitely mature, resting mature and immature. Percent frequencies total to 100% within each category.
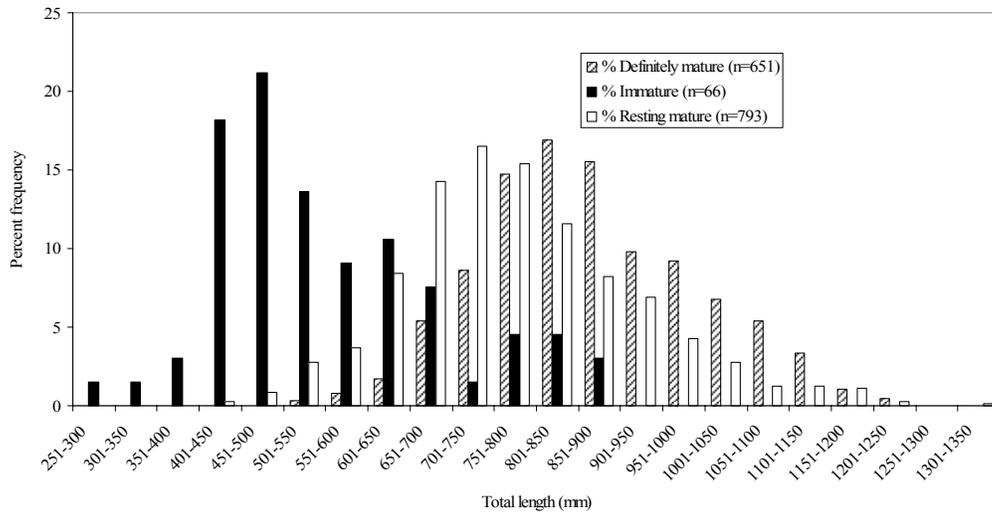
Figure 3. Size frequency histograms of gag histologically assessed and categorized as resting mature and uncertain maturity. Percent frequencies total to 100% within each category.
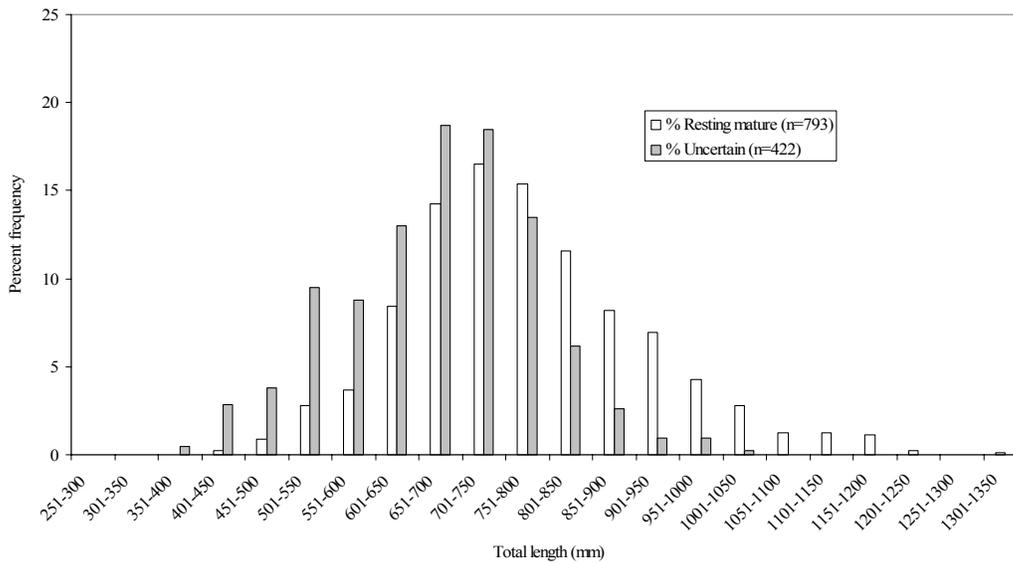
Figure 4. Length at maturity based on definitely mature and immature female gag. Logistic regression function (Gompertz): Proportion = EXP(-EXP(-(-9.02 + 0.016*TL))), r^2 (McFadden) = 0.6, n = 707, L_{50} maturity = 585 mm TL.
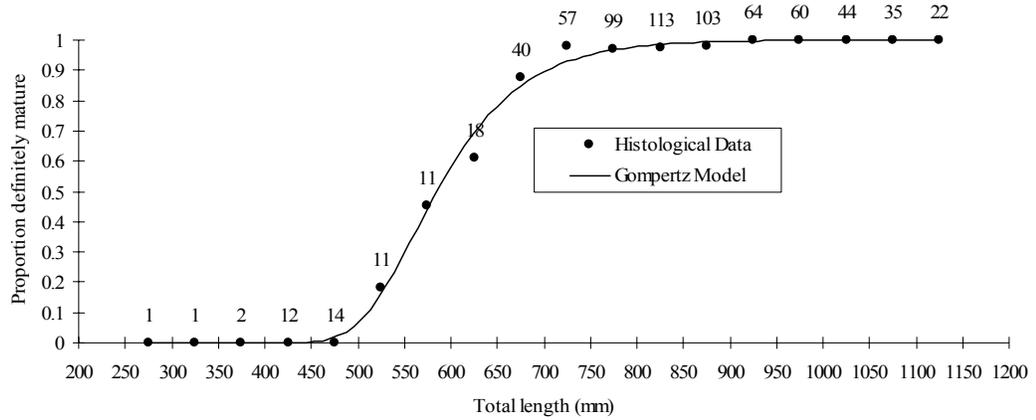
Figure 5. Age at maturity based on definitely mature and immature female gag. Logistic regression function (Gompertz): Proportion = EXP(-EXP(-(-6.42 + 1.81*Age))), r^2 (McFadden) = 0.6, n = 552, A_{50} maturity = 3.7 years.
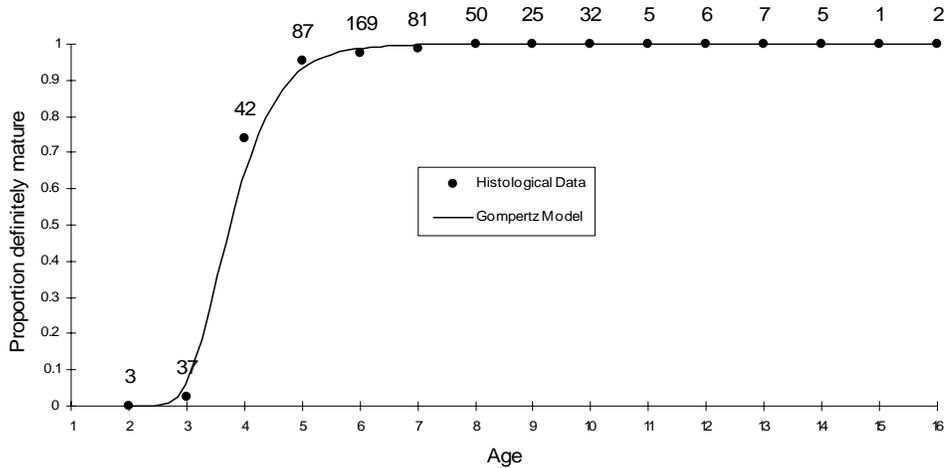
Figure 6. Estimates of spawning frequency (average interval in days between spawning events per female) by age based upon females exhibiting hydrated oocytes. Grey lines indicate the 95% confidence intervals for mean predicted values. Regression: Frequency hydrated = 0.078*Age + 3.2, $r^2 = 0.015$, mean frequency across ages = 3.7d.
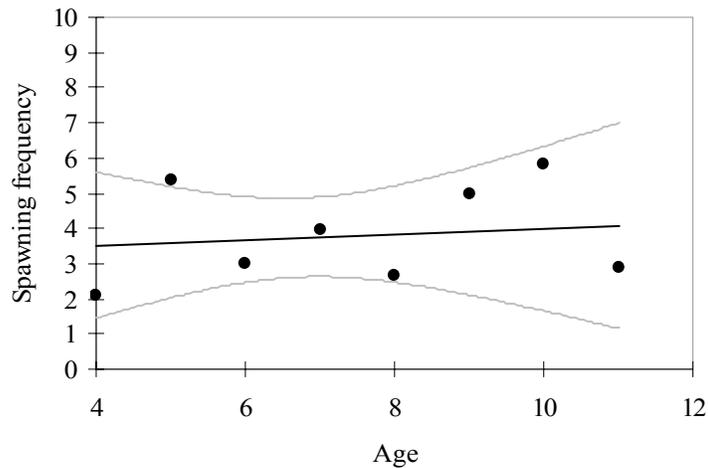
Figure 7. Batch fecundity by total length. Regression equation: Batch fecundity (thousands) = 1.669*TL – 1024, $r^2 = 0.34$, n = 70.
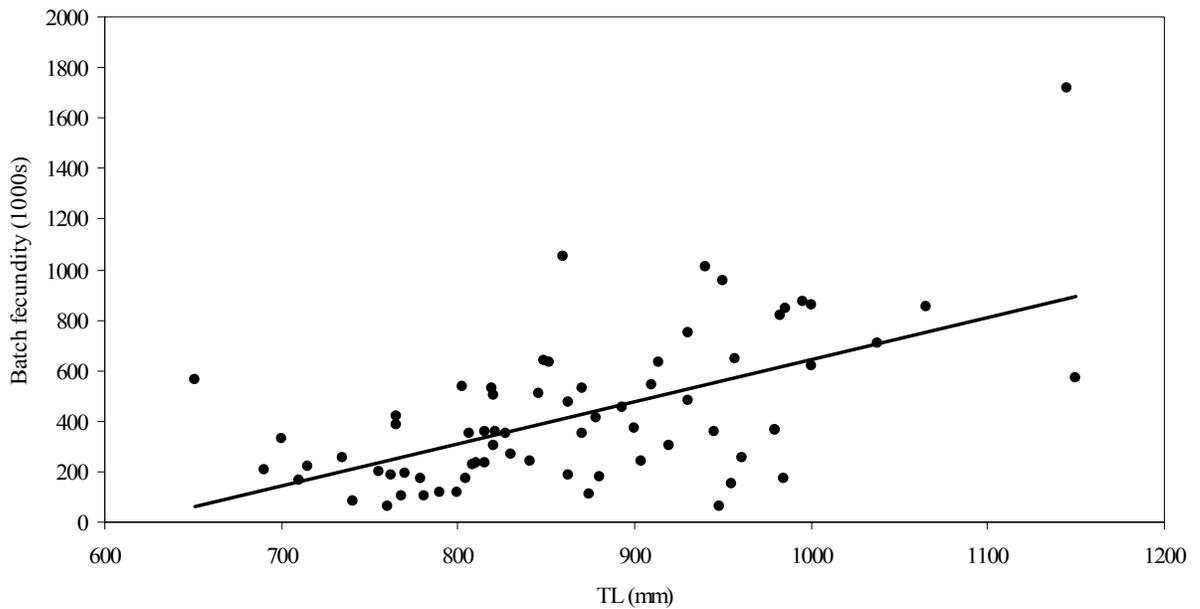
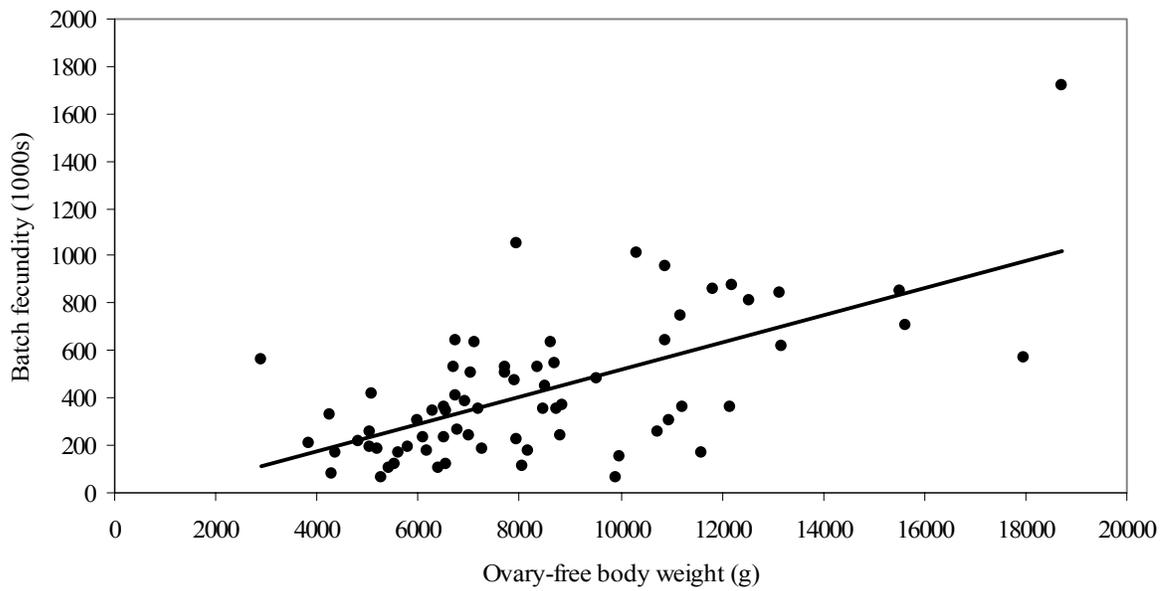Figure 8. Batch fecundity by age. Regression equation: Batch fecundity (thousands) = 80.997*Age – 151.2, $r^2 = 0.30$, n = 61.
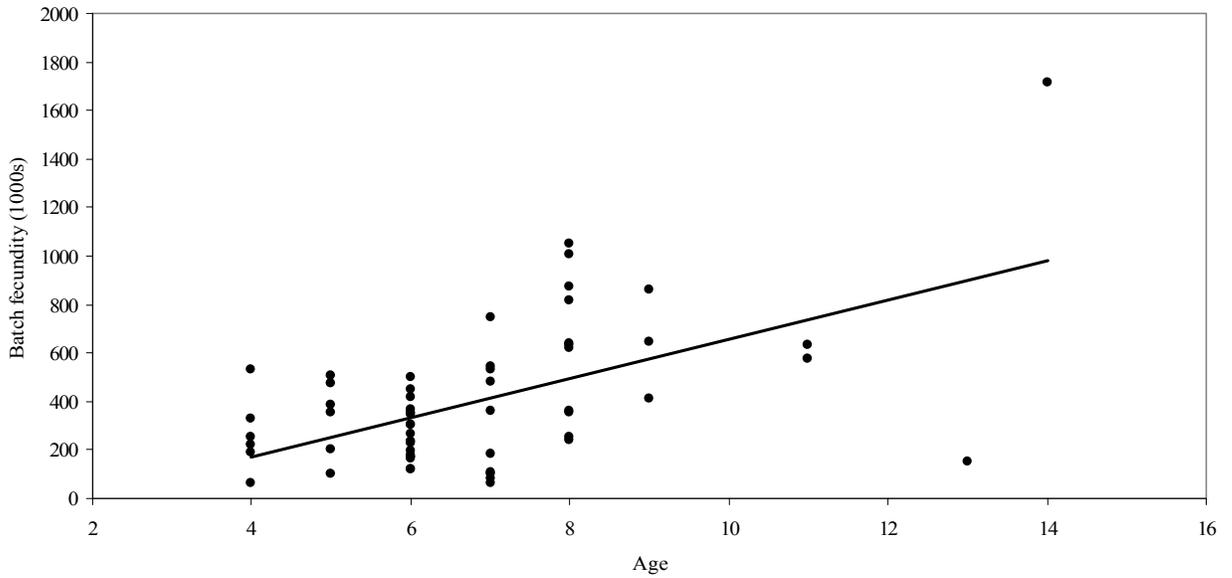
Figure 9. Batch fecundity by gonad-free body weight (Wt, g). Regression equation: Batch fecundity (thousands) = 0.058*Wt – 59.15, $r^2 = 0.40$, n = 70.

Figure 10. Proportion female by size, assessed histologically. Logistic regression function (Gompertz): Proportion = EXP(-EXP(-(14.39 -0.013*TL))), r^2 (McFadden) = 0.52, n = 2076, L_{50} transition = 1100 mm TL.
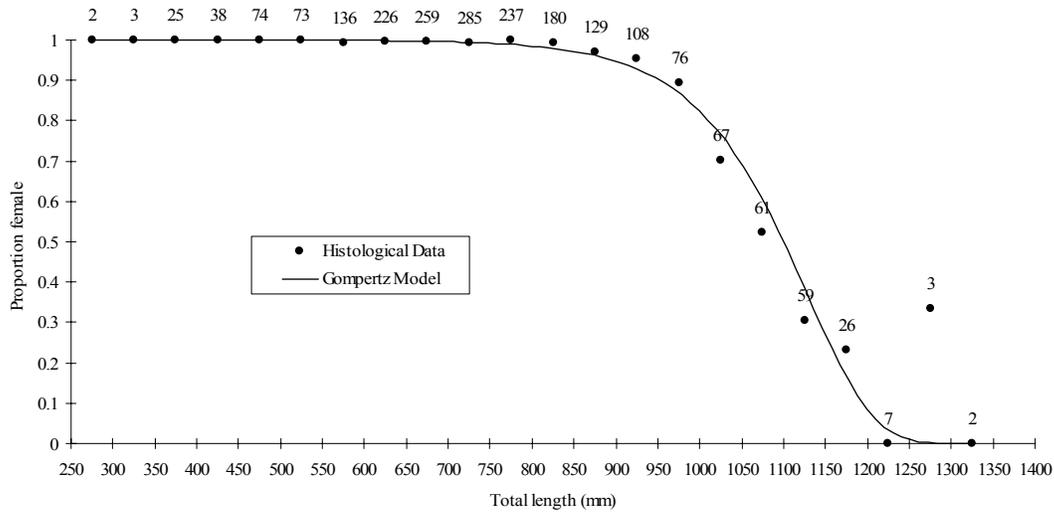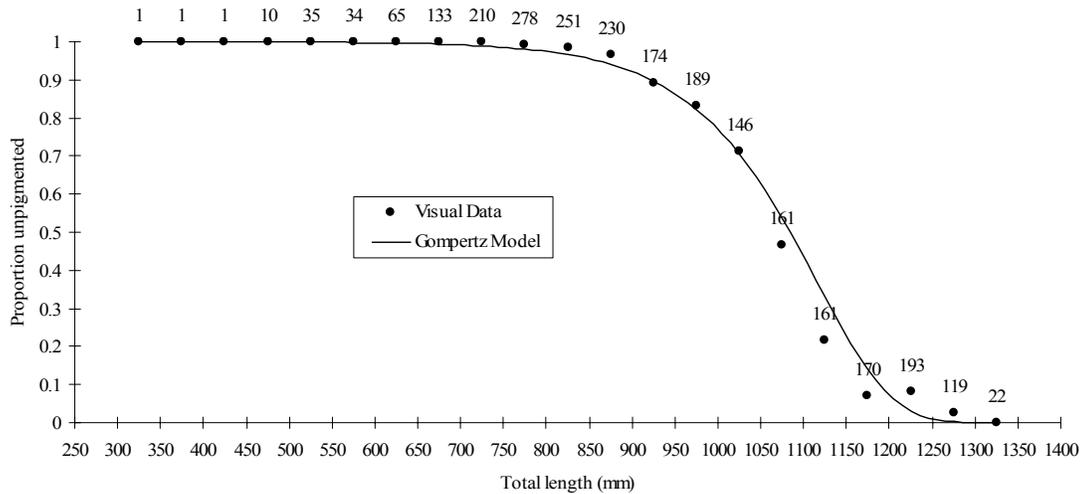

Figure 11. Proportion unpigmented (assumed female) by size assessed visually from collections containing at least one copperbelly pigmented gag. Logistic regression function (Gompertz): Proportion = EXP(-EXP(-(12.88 -0.0115*TL))), r^2 (McFadden) = 0.6, n = 2584, L_{50} = 1085 mm TL.

Figure 12. Proportion unpigmented (assumed female) by age, assessed visually from collections containing at least one copperbelly pigmented gag. Logistic regression function (Logit): Proportion $= 1 / (1 + EXP(-(6.89 -0.637*Age)))$, r^2 (McFadden) = 0.57, n = 2462, A_{50} transition = 10.8 years.