# Draft Working Document

# Yellowedge grouper (*Epinephelus flavolimbatus*) age, growth and reproduction from the northern Gulf of Mexico

Melissa Cook and Michael Hendon

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National Marine Fisheries Service Southeastern Fisheries Science Center 3500 Delwood Beach Road Panama City, Florida 32408

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# Introduction

Age, growth, and reproduction information is critical to stock assessment; the goal of this report is to characterize age-length structure and reproductive parameters using data collected from the northern Gulf of Mexico. Yellowedge grouper age, growth and reproductive characteristics were reviewed and new information was added to data previously described by Cook (2007). The following are discussed: age and length frequencies, growth rates, age and size at maturity, sexual transition, spawning season and reproductive potential or proxies for fecundity.

# Methods

# Data Collection

Yellowedge grouper otoliths and gonads were sampled for fish collected using bottom longline gear by both commercial and scientific sources, scientific trawls surveys and by commercial hand line gear. Otoliths were collected (1979-2009) by numerous federal and state sources representing the commercial fisheries (Trip Interview Program – TIP, Alabama Division of Marine Resources – ALMR). Yellowedge grouper otoliths were first collected in 1979 via a federally funded fishery independent survey (NMFS Pascagoula, MS – MSLAB), which reestablished biological sample collection of yellowedge grouper in 1999. The Cooperative Research Program (CRP) also provided otoliths and gonads and site specific detailed capture locations. Atsea collection of otoliths and gonads were made possible through two observer programs (NMFS Panama City Shark Bottom Long-line Observer Program – SBLOP and NMFS Galveston Reef fish Observer Program – GOP). Yellowedge grouper were sampled for reproductive research throughout the year from 1999-2009, although samples were not collected monthly within each year. Measurements of fish lengths (total and/or fork), weights (whole or gutted), and removal of otoliths and gonads were completed in the field. Gonads were removed and stored in 10% neutral buffered formalin until histological processing.

Information describing catch location (latitude, longitude, depth, or NMFS statistical subareas, further referred as grids; Patella 1975) was often reported with commercial samples. Depth data were either reported as a mean depth or a range of depths for the entire interview. If the range of depth was  $\leq$  5-fathoms (fm), then an average depth was calculated, otherwise both a start and an end depth were recorded.

Data Quality Control

Each of the data collection sources has separate but similar sampling procedures, data protocols, and reporting methodologies. Our facility uses data quality control guidelines in the interpretation of source-specific datasheets as described by the Procedure Manual for Age, Growth, and Reproduction (AGR) Lab (NOAA 2008). First, each species-specific collection is assigned an annual collection (or tracking) number and all collection-specific data (i.e. source, source number, state, sector, and gear) are proofed and entered in our Annual AGR Access Databases from the original datasheets. If such data are not provided, then the collector (port agent and/or survey leader) is contacted to track down the missing data. Our Annual AGR Access Databases were constructed with field-specific lists of suitable values (e.g. source, state, sector, and gear), validation rules, and user-specific security for data accessibility to enhance our data quality control procedures. Additionally, the source number (or interview number) is a source-specific number (or combination of intercept specific numbers) that permits the cross-referencing of data between databases (original source and Annual AGR Database). Next, after all the individual fish data are entered, proofing sheets are reviewed against the original datasheets and any corrections are made to the Annual AGR Database. Finally, all proofing sheets are initialed, dated and filed for further reference. Prior to 1998, no manual existed to implement these procedures. Therefore, to insure these standards of quality control, all 1991-1997 data were proofed using the TIP original datasheets (archived in Panama City, FL) and any missing data were resolved by accessing the TIP database (DELPHI, SEFHOST).

# Age and Length Analysis

Not all yellowedge grouper otoliths were selected for ageing. Due to the large number of otoliths collected from the Trip Interview Program (TIP) during 2001, 2003, 2007-2009, otoliths were subsampled for ageing. Only otoliths collected in the state of Florida from fish harvested by the commercial longline sector were subsampled. To obtain an accurate representation of the catch, otoliths were subsampled by grid based on the commercial longline landings data. Not all otoliths collected in 1993, 1998 and 2000 were aged because some were lost in Hurricane Katrina before they could be aged.

Sagittal otoliths were extracted and ages were determined using the methods of Cook (2007). Each otolith was independently aged readers who lacked knowledge of specimen length or

date of capture. Due to difficulty experienced by previous investigators otoliths were assigned a readability based on Kuo and Tanaka (1984) (Table 1). Two readers aged yellowedge grouper and indices of precision (Percent Agreement, Average Percent Error and CV) were calculated. All fish were assigned an annual age equal to the annulus count by convention.

Age and length data and observed mean size-at-age data are presented for fishery dependent and fishery independent data. Results are presented as predicted total length (TL). If total length was not available, fork length (FL) was converted to TL using the following equation: Predicted TL = 1.067\*FL - 15.065,  $R^2 = 0.997$ , n=1,593). In order to determine general biological growth parameters all age data were combined regardless of sector and gear type used. A growth curve, based on ages and predicted total lengths at capture, was modeled using the von Bertalanffy growth function and was fit by non-linear regression (Solver, Microsoft Excel). Data were grouped into 100-m depth bins to evaluate depth of capture. Commercial longline data was also compared by NMFS grid to identify any patterns of length, age, or size-at-age among the capture locations.

# **Reproductive Analysis**

All gonads were removed from formalin, blotted dry, and weighed to the nearest 0.1 g. The posterior of one or both lobes of the gonad was cross sectioned and submitted for histological processing (Cook 2007). 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. Histological slides were examined microscopically at 40x - 400x magnification to determine oocyte maturation. Using the oocyte maturation characteristics described by Lyon et al. (2008), oocytes were staged accordingly to determine the leading oocyte stage, presence or absence of postovulatory follicles (POFs) and gonad class.

Spawning Season. The gonadosomatic index (GSI) was calculated for males and females using the following formula: GSI = (GW/(TW-GW)) \* 100; where GW = total fresh gonad weight (g) and TW = total fish weight (g). The value of TW minus GW is also referred to as "somatic weight" for purposes of comparison.

*Size and age at maturity and sexual transition.* All records of maturity were based upon histological examination. Females displaying vitellogenic or hydrated oocytes were defined as mature. Females were classified as "spawning" depending upon the presence of hydrated oocytes, indicative of imminent spawning, or POFs, indicative of recent spawning (Hunter and Macewicz

1985). Females with cortical alveoli or primary growth oocytes as the leading stage and with evidence of prior spawning (old, atretic hydrated oocytes, muscle bundles, brown bodies, etc.) were also classified as mature females. Regressed and developing females with few prior indicators of spawning were excluded from the analysis because their maturity was uncertain. A logistic regression model fitted to binomial maturity data (immature=0, mature=1) was used to determine the size and age at which 50% of females in the population reached sexual maturity. The analysis was conducted using females collected during all months of the year. A logistic regression model fitted to binomial data (female=0, male=1) was used to determine the size and age at which 50% of females.

*Fecundity Proxy.* Fecundity analysis was not conducted due to the small number of hydrated gonads available (all gonads collected from 1999-2005 were lost in Hurricane Katrina). However, the relationship between gonad weight and age is presented for comparison. Only females with vitellogenic or hydrated oocytes collected during the peak spawning months (March through September) were used. Ovary weight relationships were compared with somatic weight at age; which in turn is commonly extrapolated by numbers at age to yield estimates of spawning stock biomass (SSB).

# Results & Discussion

# Age and Length Sample Sources

A total of 10,417 yellowedge grouper were collected and sampled from 1979-2009. A subsample of 8,197 otoliths were selected for ageing. The following results only pertain to fish which were successfully aged (n=7,394). Although yellowedge grouper were collected over a thirty year time period, sampling effort was not evenly distributed temporally, and varied considerably by sector and gear which made comparable comparisons over time difficult. Ninety-four percent of the yellowedge grouper otoliths were collected during the more recent years (1998-2009). The majority of samples came from the west coast of Florida (63%), followed by Louisiana (20%), Texas (15%), Mississippi (<1%) and Alabama (<1%) (Table 2). The bulk of samples were obtained from the trip interview program (TIP; 83%), fishery independent surveys (7%), cooperative research programs (5%) and scientific observer programs (4%) (Table 3). Yellowedge

grouper otoliths were mainly collected from fish harvested in the commercial longline (76%) and hand line fisheries (16%), and scientific longline (6%) and trawl surveys (1%) (Table 4).

# Age Determination

Ages were determined for 90% (n=7,394) of otoliths viewed. The majority of otoliths were classified as readable (65%) according to the readability scale. Remaining otoliths were classified as good (3%) or difficult (22%). Only 8% otoliths were considered unreadable and 2% (n=153) were excluded due to breakage or preparation problems (Table 5). The cumulative percent of readings in exact agreement between both readers was 16.8%, agreement ±1 was 44.1%, and agreement ±2 was 63.2% while percent agreement increased to 91.9% ±5 years. Differences of greater than five years occurred for only 8.10% of otoliths aged. The overall APE for all yellowedge grouper aged was 9.07% with a CV of 12.83%. The latest results indicated an increase in reader precision as readers gained experience. The APE reported by Cook (2007) was 11.9% (CV=16.8%) and percent agreement was only 78.5% ±5 years, suggesting increased precision for recently collected and aged otoliths.

# Description of Age, Length and Growth

Yellowedge grouper ranged from 100-1,228 mm TL (mean=656, SE=1.82) (Figure 1 A) and ages 0-85 years (mean=14.9, SE=0.10) (Figure 1 B). A summary of descriptive statistics by time period): 1) 1979-1989, 2) 1991-1994 and 3) 1998-2009), sector and gear is presented in Table 6. Observed mean size-at-age data are presented for commercial hand line (1991-94 vs. 1998-09, Figure 2) and commercial hand line vs. commercial longline (1998-2009, Figure 3). Yellowedge grouper harvested using hand lines were slightly larger and older during 1991-94 (mean=684 mm TL, mean=18 years) than during 1998-2009 (mean=636 mm TL, mean=13 years). Commercial longline gear captured larger and older fish (mean=661 mm TL, mean=15 years) than commercial hand line gear (mean=636 mm TL, mean=13 years).

Von Bertalanffy growth curves were fitted to total length at age data for all yellowedge grouper combined (Figure 4) and each time period (Figure 5; Table 7). Overall growth (*K*) was slow (*K*=0.059) resulting in a theoretical asymptotic length ( $L_{\infty}$ ) of 1,005 mm TL. Yellowedge grouper growth rate appeared to increase slightly from 1979-1989 (*K*=0.042) to 1991-1994 (*K*=0.059) but remained comparable through 1998-2009 (*K*=0.058).

Yellowedge grouper were collected throughout the GOM between 30-350 m depths (mean=166 m) (Figure 6). The NMFS statistical grids were used to evaluate spatial patterns. The majority of aged yellowedge were collected in grid four followed by grids three and eight (Figure 7). Since commercial longline gear composes the majority of the harvest, age and length data was evaluated by grid (Figure 8 A, B). Yellowedge grouper were larger and slightly older in the western GOM (Table 8). Grid 17 had the largest and oldest averages per grid but only 1% of fish were collected there. The smallest and youngest fish were collected in grids seven and ten, respectively.

# **Reproductive Sample Sources**

Gonad and otoliths were collected from a total of 942 yellowedge grouper from the northern GOM from 1999-2009 (Table 9). This included an additional 337 fish which were added to the dataset presented by Cook (2007). Yellowedge grouper were primarily collected from Florida waters (Figure 9). The majority of samples (98%) were collected using bottom longline gear by both commercial and scientific sources. The remaining 2% of samples were collected on scientific trawls surveys and by commercial hand line gear (Figure 10).

# Reproductive and age summary

Females (n=712) ranged from 141 – 1000 mm TL (mean=638, SE=4.7) while males (n=221) ranged from 412 – 1228 mm TL (mean=832, SE=7.8). Transitional fish (n=7) ranged from 635-840 mm TL (mean=733, SE=25.6) (Figure 11). Reproductive and age (range 1-70 years) information was available for 940 yellowedge grouper. Mean ages of females (13 years, SE=0.2, range 1-36 years) were considerably lower than males (23 years, range 5-70 years, SE=0.6) and transitional fish (16 years, SE=2.6, range 9-31 years). The overall sex ratio for yellowedge grouper sampled was 1:3.2 (male:female). The additional data resulted in very little to no change from results initially described in Cook (2007).

Spawning season. Yellowedge grouper displayed a prolonged spawning season. Females in active (gonads containing vitellogenic oocytes, n=216) or spawning (gonads containing hydrated oocytes, n=62) condition were observed from February through September and in November (Figure 12). Peak spawning months occurred in March through September. GSI values were the highest in March and in July through September (Figure 13) and displayed a bimodal spawning

season. Monthly sample size and annual spawning variability could explain the bimodal distribution.

*Size and age at maturity and transition.* Histological examination of yellowedge grouper ovaries revealed all sexual maturation stages, from immature through regressed were present. Immature females primarily ranged in age from 0-16 years old and 141-650 mm TL (one outlier of 810 mm TL, 17 years was observed) (Figure 14). Most females began reaching sexual maturity at age six and were sexually mature by age seventeen. Mature females primarily ranged from 510-1000 mm TL, ages 6-36 years (one outlier of 454 mm TL, 10 years was observed).

Female length and age at 50% percent maturity were 547 mm TL (95% CI=462-672) and 8 years (95% CI=7-10), respectively (Figures 15, 16). Cook (2007) found that size and age of 50% maturity of active females was 543 mm TL and 8 years, respectively. Size and age at 50% sexual transition for GOM yellowedge grouper was 815 mm TL (95% CI=706-951) and 22 years (95% CI=19-26) (Figures 17, 18). Results were similar to those reported by Cook (2007) for the eastern GOM. Similar results are not atypical since the majority of new data was from the eastern GOM.

*Fecundity Proxy.* Based upon histologically sexed yellowedge grouper, 265 females were available to estimate average somatic weight at age (Figure 19). Active and spawning yellowedge grouper females ranged in age from 6-36 years old, the majority (87%) were twenty years old and younger. The relationships between hydrated and vitellogenic ovary weight and somatic weight were fairly proportional when graphically compared, these data (extrapolated to SSB) may be selected as the proxy for fecundity.

The objective of this study was to provide additional age and growth information as well as updated information on spawning season, size and age at sexual maturity, and sexual transition for GOM yellowedge grouper. Overall, demographic results are similar to earlier findings reported by Cook (2007).

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Table 1. Readability classifications assigned to yellowedge grouper otoliths. Classification scale based on Kau and Tanaka (1984).

Category	Description
Good	The opaque and the hyaline zones are separated distinctly; growth increment number can be read easily.
Readable	Although the opaque and the hyaline zones are not separated so distinctly, growth increment number can be read.
Difficult	The opaque and hyaline zones are difficult to distinguish, and usually the third reading is required to decide the growth increment number.
Unreadable	The opaque and the hyaline zones can not be distinguished, and the growth increment number is regarded as uncertain.
Preparation	The otolith was not aged due to breakage or preparation (Ex. otolith damaged and pieces too small to section or section missed the core).

Year	FL	AL	MS	LA	TX	Total
1979	6					6
1982	1	12				13
1983				25		25
1984					29	29
1985	8					8
1986					21	21
1987					3	3
1988					9	9
1989					5	5
1991				247	2	249
1992	11			58		69
1993				9		9
1994				2		2
1998	5					5
1999	57	33	7			97
2000	98	3	6	6	25	138
2001	384	5	1	34	15	439
2002	177	1	17	40	3	238
2003	781	2	9	14	8	814
2004	494			34	53	581
2005	556		1	91	33	681
2006	302		10	105	61	478
2007	543	1	1	99	223	867
2008	605	1		302	366	1274
2009	617	5		420	288	1330
Total	4645	63	52	1486	1144	7390
Percent	63%	<1%	<1%	20%	15%	

Table 2. Summary of the number of yellowedge grouper otoliths aged by state landed (FL – west coast Florida, AL – Alabama, MS – Mississippi, LA – Louisiana, TX - Texas).

Year	TIP	MSLAB	CO-OP	SBLOP	HB	Total
1979		6				6
1982		13				13
1983		25				25
1984		29				29
1985		8				8
1986					25	25
1987					3	3
1988					9	9
1989					5	5
1991	247				2	249
1992	58					58
1994	2					2
1998	5					5
1999	55	41				96
2000	103	35				138
2001	402	37				439
2002	191	47				238
2003	581	62	170			813
2004	422	47	112			581
2005	595	23	63			681
2006	429	48				477
2007	799	37		31		867
2008	956	22	2	294		1274
2009	1275	39	16			1330
Total	6120	519	363	325	44	7371
Percent	83%	7%	5%	4%	<1%	

Table 3. Summary of the number of yellowedge grouper otoliths aged by source (TIP - Trip Interview Program, MSLAB - NMFS Pascagoula MS, CO-OP - Cooperative Research Proposals, SBLOP – Shark Bottom Long-line Survey, HB – Beaufort Head Boat).

Table 4. Annual number of yellowedge grouper otoliths aged by sector (CM - Commercial, SS -
Scientific Survey, HB - Head Boat) and gear (LL - Longline, HL - Hand Line, TRW - Trawl).
Other refers to fish collected by charter party, head boat, private, scientific hand lines; scientific
trap.

Year	CM	CM	SS	SS	HB	Other	Total
	LL	HL	LL	TRW	HL		
1979			6				6
1982			13				13
1983			25				25
1984			29				29
1985			8				8
1986					25		25
1987					3		3
1988					9		9
1989					5		5
1991	12	212			2	23	249
1992	38	31					69
1993	3	6					9
1994		2					2
1998	5						5
1999	55		41			1	97
2000	90	13	29	6			138
2001	350	52	28	8		1	439
2002	152	39	40	6		1	238
2003	704	47	55	7		1	814
2004	493	41	37	10			581
2005	580	78	10	11		2	681
2006	376	53	34	14		1	478
2007	714	116	31	6			867
2008	1009	243	9	12		1	1274
2009	1010	281	36	3			1330
Total	5591	1214	431	83	44	31	7394
Percent	76%	16%	6%	1%	<1%	<1%	

Table 5. Number of aged yellowedge grouper based on otolith readability scale of Kau and Tanaka (1984).

Readability	Total	Percent
Good	209	3%
Readable	5350	65%
Difficult	1835	22%
Unreadable	650	8%
Preparation	153	2%
Total	8197	

Table 6. Summary of life history statistics for yellowedge grouper from the northern Gulf of Mexico. Yellowedge grouper were collected in 1979-1989 (n=123), 1991-1994 (n=327) and 1998-2009 (n=6,934) by head boat (HB), scientific survey (SS), commercial (CM) and charter party (CP) sectors using hand line (HL), bottom longline (LL), and trawl (TRW) gear types. Results include the sample size (n), range (minimum-maximum), mean, standard deviation and standard error for each parameter: total length (mm) and age (years).

Time Period	Mode Gear	Parameter	п	Range (Min-Max)	Mean	Standard Deviation	Standard Error
1979-1989	HB HL	Total Length	42	335-710	493.55	87.14	13.45
		Age	42	4-11	5.60	1.75	0.27
	SS LL	Total Length Age	81 81	488-1050 5-81	735.33 25.59	136.94 16.14	15.22 1.79
1991-1994	CM HL	Total Length Age	251 251	290-1110 2-70	684.47 17.86	162.61 13.76	10.26 0.87
	CM LL	Total Length Age	53 53	460-1100 3-50	706.81 14.83	138.09 8.19	18.97 1.13
	CP HL	Total Length Age	23 23	425-1160 5-77	789.57 22.96	173.86 20.55	36.25 4.28
1998-2009	CM HL	Total Length Age	963 963	262-1092 2-52	635.50 13.21	141.49 6.53	4.56 0.21
	CM LL	Total Length Age	5538 5538	211-1178 1-85	660.72 15.13	147.74 7.88	1.99 0.11
	SS LL	Total Length Age	350 350	322-1228 2-70	703.84 15.90	165.79 9.09	8.86 0.49
	SS TRW	Total Length Age	83 83	100-1075 0-38	219.54 2.70	157.47 6.03	17.28 0.66

Table 7. Results of yellowedge grouper von Bertalanffy growth curves from fish collected throughout the northern Gulf of Mexico during 1979-2009. Source refers to the data used in the analysis, predicted TL is total length, n is the number of samples,  $L_{\infty}$  is the maximum theoretical length, K is the growth coefficient,  $t_0$  is the theoretical age at length zero,  $R^2$  is the coefficient of determination.

Source	Size Range Examined (TL mm)	Age Range Examined (Years)	п	$L_{\infty}$	K	$t_0$	$R^2$
All years	100-1,228	0-85	7394	1,004.5	0.059	-4.75	0.68
1979-1989	335-1,050	4-81	123	966.9	0.042	-11.87	0.67
1991-1994	290-1,160	2-77	329	969.8	0.059	-7.452	0.72
1998-2009	100-1,228	0-85	6942	1,017.7	0.058	-4.576	0.68

Grid	Sample	Mean	Mean Age
	Size	Total Length	(years)
		(mm)	
2	63	673.76	16.8
3	597	643.66	15.7
4	1585	644.98	15.6
5	551	615.15 **	13.4**
6	182	665.14	14.2*
7	29	587.31**	11.5**
8	619	634.42*	13.5**
9	172	718.27*	14.9
10	121	604.34**	9.9***
11	42	703.56	13.8
13	48	641.73	13.9
14	164	719.24*	17.1
15	51	670.63	14.8
16	64	781.50***	18.5
17	10	883.40***	28.9***
18	17	653.76	14.1
19	84	654.67	14.6
20	440	716.35*	16.6
21	446	745.75***	16.9
Total	5285		

Table 8. Mean total length and age by statistical grid of yellowedge grouper collected from 1998-1999 using commercial longline gear. Statistically different values are indicated (\* = 0.05, \*\* = 0.01, \*\*\* = 0.001).

Table 9. Number of yellowedge grouper gonads collected per year.

Year	Total
1999	40
2000	28
2001	32
2002	42
2003	238
2004	145
2005	82
2006	31
2007	58
2008	192
2009	52
Total	940



Figure 1. A) Length and B) age frequency distributions of yellowedge grouper collected during 1979-2009 by fishery dependent and independent sources using various gear types (bottom longline, hand line, trawls) in the northern Gulf of Mexico.



Figure 2. Comparison of yellowedge grouper commercial hand line mean size at age ( $\pm$  standard error) during 1991-1994 and 1998-2009.



Figure 3. Comparison of yellowedge grouper commercial hand line and commercial longline line mean size at age ( $\pm$  standard error) during 1998-2009.



Figure 4. Age and growth of yellowedge grouper collected from 1979-2009. The sold line represents the data fitted to the von Bertalanffy growth curve.



Figure 5. Results of von Bertalanffy growth curves for yellowedge grouper collected 1997-2009.



Figure 6. Yellowedge grouper depth of capture.



Figure 7. Percent of aged yellowedge grouper per NMFS statistical grid.



Figure 8. A.) Age and B.) length of yellowedge grouper collected during 1999-2009 by commercial longline gear.



Figure 9. Gulf of Mexico yellowedge grouper (*n*=940) collected 1999-2009 by state.



Figure 10. Gulf of Mexico yellowedge grouper (n=940) collected 1999-2009 by fishing mode and gear.



Figure 11. Scatterplot of predicted total length at age of yellowedge grouper males, females and transitional fish.



Figure 12. Percent of yellowedge grouper females classified as inactive (n=425) or active/spawning (n=287) per month. Females with vitellogenic or hydrated oocytes were considered active all other females were considered inactive. Sample size presented in bold.



Figure 13. Yellowedge grouper monthly mean Gonadosomatic Index (GSI) values for males and females. Error bars represent  $\pm 1$  standard error. All reproductive classes are included.



Figure 14. Length-frequency of immature and mature female yellowedge grouper collected during 1999-2009. Females of uncertain sexual maturity were excluded from logistic fits of maturity.



Figure 15. Length at maturity based on mature and immature female yellowedge grouper during all months of the year. Logistic regression function: Proportion = EXP(-EXP(-(-18.11 + 0.033\*Length))), n=608, L50 maturity = 547 mm TL.



Figure 16. Age at maturity based on mature and immature female yellowedge grouper during all months of the year. Logistic regression function: Proportion = EXP(-EXP(-(-3.718 + 0.451\*Age))), n=608, A50 maturity = 8.2 years.



Figure 17. Proportion female by size, assessed histologically. Logistic regression function: Proportion = EXP(-EXP(-(-11.894 + 0.015\*Length))), n=933, L50 transition = 815 mm TL.



Figure 18. Proportion female by age, assessed histologically. Logistic regression function: Proportion = EXP(-EXP(-(-4.970 + 0.223\*Age))), n=933, A50 transition = 22.3 years.



Figure 19. Mean gonad weight at age of yellowedge grouper females with vitellogenic or hydrated ova and mean somatic weight at age.