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POPULATION DYNAMICS, STRUCTURE AND PER-RECRUIT ANALYSES OF YELLOWEDGE GROUPER, *EPINEPHELUS FLAVOLIMBATUS*, FROM THE NORTHERN GULF OF MEXICO

by

Melissa Cook

Abstract of a Dissertation
Submitted to the Graduate Studies Office
of The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

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ABSTRACT

POPULATION DYNAMICS, STRUCTURE AND PER-RECRUIT ANALYSES OF YELLOWEDGE GROUPER, *EPINEPHELUS FLAVOLIMBATUS*, FROM THE NORTHERN GULF OF MEXICO

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Age, growth and reproduction research was conducted on yellowedge grouper, *Epinephelus flavolimbatus*, obtained from the commercial harvest and National Marine Fisheries Service scientific cruises in the Gulf of Mexico (GOM) during 1979-2005. Fish ranged in size from 107-1,170 mm total length (TL). Ages ranged from 0 to 85 years; maximum age greatly exceeded previously reported ages. Bomb-produced ^{14}C was used to validate ages determined by counting otolith growth increments. A strong linear relationship existed between otolith weight and fish age ($R^2 = 0.84$). The von Bertalanffy growth equation was $TL=970.8(1-e^{-0.063(t+4.84)})$. Length and age structure yellowedge grouper harvested today are considerably smaller and younger than those in the past. Yellowedge grouper are monandric protogynous hermaphrodites and have an extended spawning season from March through September. Results indicated a change in the sex ratio over the last 25 years due to a 14% decrease of males. Size at sexual maturity and size at transition have also decreased.

Yellowedge grouper were distributed throughout the GOM, but regional differences in population density, size and age structure and sexual maturity suggest at least two or three separate stocks occur in the GOM. Yellowedge grouper in the western GOM were larger, older and more abundant while fish in the eastern GOM were smaller

and younger. Yellowedge grouper in the eastern GOM aggregate in denser patches than those in the western GOM.

Yield-per-recruit and spawning stock biomass-per-recruit analyses were applied to determine biological reference points and evaluate the status of the fishery. Results indicated stocks cannot sustain high levels of fishing mortality (F<0.10). Yellowedge grouper are currently experiencing growth overfishing but not recruitment overfishing. The importance of male spawning stock biomass-per-recruit was demonstrated and is suggested as an additional tool for use by managers to avoid stock collapse when managing a protogynous hermaphrodite. Significant changes in the age, length and sex structure were found, particularly in the eastern GOM, since the onset of commercial fishing. Continued monitoring of life history parameters is necessary to ensure continued survival of this species in the GOM.

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LIST OF ABBREVIATIONS

AMS……Accelerator Mass Spectrometry

ANCOVA……..Analysis of covariance

ANOVA……Analysis of variance

APE…….Average percent error

C…….Central

CI……Confidence interval

CPUE……Catch-per-unit-effort

CV……Coefficient of variation

E…….Eastern

F……Instantaneous rate of fishing mortality

FL……....Fork length

FMP……..Fishery Management Plan

FOM…….Final oocyte maturation

g……Gram

GMFMC…….Gulf of Mexico Fisheries Management Council

GOM…….Gulf of Mexico

GSI……..Gonadosomatic Index

GW……..Gonad weight

K……..…von Bertalanffy growth coefficient

kg……Kilogram

K-S……...Kolmogorov-Smirnov two-sample test

L_{∞}……..Maximum theoretical length

LRT……Likelihood ratio test

M……...Instantaneous rate of natural mortality

m……...Meter

mm……Millimeter

MSFCMA……..Magnuson-Steven Fishery Conservation and Management Act

n……..Number of samples

MS Lab…….Pascagoula, Mississippi Laboratory

MSP……..Maximum spawning potential

NBF……..Neutral buffered formalin

NMFS……..National Marine Fisheries Service

NOAA……..National Oceanic and Atmospheric Administration

NOSAMS……...National Ocean Sciences Accelerator Mass Spectrometry

POF……Postovulatory follicles

RFRFMP……..Reef Fish Resources of the Gulf of Mexico Fisheries Management Plan

SEAMAP……..Southeast Area Monitoring and Assessment Program

SEDAR……South East Data, Assessment and Review

SPR…….Spawning potential ratio

SSB/R……...Spawning stock biomass-per-recruit

t_0……..Theoretical age at length zero

TIP……....Trip Interview Program

TL……...Total length

W……..Western

W_∞……Maximum theoretical weight

WHOI……...……..Woods Hole Oceanographic Institute

YPR……..…Yield-per-recruit

Z……Instantaneous rate of total mortality

CHAPTER I

INTRODUCTION

The yellowedge grouper (*Epinephelus flavolimbatus,* Poey 1865) is a member of the family Serranidae and subfamily Epinephelinae. This species is found in the western Atlantic from North Carolina (Huntsman, 1976) to southern Florida, the Gulf of Mexico (GOM), Cuba (Smith, 1971), the West Indies, off the coasts of Central America, and the northern coast of South America to Brazil (Carpenter and Nelson, 1971; Smith, 1971; Carpenter, 2002). Yellowedge grouper inhabit moderately deep water and typically occur at depths between 90-365 m (Smith, 1971). Unlike most grouper, which are associated with reefs and structure, yellowedge grouper can be found in a variety of habitats. In Texas they are often found over areas of flat bottom, near "lumps" associated with tilefish, *Lopholatilus chamaeleonticeps*, and rock ridge habitats (Roe, 1976; Jones et al., 1989). In the western GOM, yellowedge have been observed in three distinct types of burrows cut into soft sediment at depths of approximately 275 m (Jones et al., 1989). They have also been found at the shelf edge on mud, sand or sand-shell bottom (Jones et al., 1989; Heemstra and Randall, 1993). Smith (1971) reported juveniles in depths as shallow as 33 m and National Marine Fisheries Service (NMFS) trawling surveys have captured juveniles from 13-115 m. Eggs and larvae are pelagic and cannot be distinguished from larval snowy grouper, *Epinephelus niveatus,* therefore, no early life history information is known for yellowedge grouper (Richards, 1999).

Yellowedge grouper are a large fish with a robust reddish brown body. They reach a maximum size of 1,150 mm total length (TL) and can weigh up to 14 kg (Heemstra and Randall, 1993). Defining taxonomic characters include: ctenoid scales, a

single dorsal fin with 11 spines and 14-15 soft rays; 3 anal fin spines and 9 rays; pectoral fin with 17-19 rays; 23-26 gill rakers, and 3 opercle spines (Smith, 1971; Carpenter, 2002). Yellowedge grouper resemble snowy grouper but are easily distinguished by their bright yellow iris and yellow fin margins (Bullock and Smith, 1991). A distinct pearly blue line runs from the eye to the angle of the preopercle. Juveniles display rows of pearly white spots and have a saddle at the top of the caudal peduncle that does not extend below the lateral line as it does in young snowy grouper (Smith, 1971; Carpenter, 2002). Live adults (> 800 mm) have also been observed displaying a spotted pattern; however, the spots fade within minutes of removal from the water (Bullock and Smith, 1991; Cook, personal observation).

Yellowedge grouper represent an important part of the multi-species deep-water grouper commercial fishery. The introduction of large-scale commercial longlining beginning in the early 1980's greatly increased commercial harvest of yellowedge grouper in the GOM (Bullock and Smith, 1991). There are incidental captures recorded in the recreational fishery, but this species is not targeted probably because of the distance from shore required (>120 km) to reach depths where yellowedge grouper usually occur. Prytherch (1983) described the longline fishery of the GOM as having three major fishing areas; the Eastern Gulf (St. Petersburg, FL area), Northern Gulf (Panama City, FL area) and the Western Gulf (Texas area). According to Prytherch (1983), yellowedge grouper was the most abundant species taken on commercial longlining vessels surveyed. However, commercial landings have only been recorded by individual species since 1985. Yellowedge grouper have been the third most abundant grouper harvested in the GOM since 1991 and the most abundant deep-water grouper harvested since 1985

(Personal communication from the National Marine Fisheries Service, Fisheries Statistics and Economics Division, Silver Spring, MD). Despite its importance as a commercially harvested species there is a lack of available life history information as well as information on distribution, abundance, mortality and status of the population.

Therefore, the initial objectives of this study were to determine age, growth and reproductive information of the yellowedge grouper. These data included the current length and age structure in the GOM, spawning season, spawning frequency and sex ratio. Comparisons were also made between results from this study and previous life history studies to determine if changes occurred over time. Fishery independent data was used to determine distribution, habitat preferences and abundance throughout the GOM and to evaluate if multiple stocks occur in the GOM. Results were then applied to yield-per-recruit and spawning stock biomass-per-recruit fisheries models to evaluate the impacts of fishing, determine biological reference points and provide management recommendations.

CHAPTER II

AGE, GROWTH AND REPRODUCTION

Introduction

Hard body parts such as scales, otoliths, fin rays, spines or bones are commonly used for ageing fish. These techniques are used because regular growth markings are incorporated in hard parts to which a time scale can be assigned, typically days or years. Otoliths are one of the most commonly used hard parts for ageing grouper. Otoliths are located in the inner-ear, paired labyrinth systems near the brain and provide sensory input needed for orientation, balance and the detection of sound. Fish have three pairs of otoliths comprised of the sagittae (the largest and most often used for ageing), lapillae, and asteriscae. Otoliths are composed of calcium carbonate crystals embedded in an organic matrix. Growth increments within otoliths are typically viewed as alternating opaque and translucent increments using transmitted light. Increment formation begins around the primordium (nucleus) of the otolith with concentric layers deposited as the fish grows. Translucent growth increments appear light in color whereas opaque increments are dark. The combination of one dark and light increment typically indicates one year of growth, an annulus (Nielsen and Johnson, 1983). Mark-recapture of chemically-tagged individuals is the most accurate method to confirm the frequency of increment formation, but this method is time consuming, expensive, and does not provide absolute ages. Marginal increment analysis has also been used to confirm annual growth increment formation.

A recent finding determined that the atmospheric nuclear bomb testing in the 1950s and 1960s left a dated mark on otoliths, and thus can provide a method to validate

annuli and determine accurate, absolute ages of long-lived fish (Kalish, 1993; Campana, 1997). Radiocarbon (^{14}C) is produced naturally in the atmosphere by the interaction of cosmic rays and nitrogen atoms. Once formed, the ^{14}C rapidly combines with oxygen to produce $^{14}CO_2$ which is mixed throughout the atmosphere and dissolved in the oceans (Kalish, 1993). Prior to the 1950's a balance existed between the input of $^{14}CO_2$ to the ocean and the production of ^{14}C in the atmosphere. However, from July 16, 1945 to November 4, 1962 the United States detonated over 300 atmospheric nuclear bombs and an unknown number of bombs were detonated by other nations around the world. A treaty signed on August 5, 1963, by the United States and Soviet Union banned the testing of nuclear weapons in the oceans, atmosphere and space but testing still continued in underground locations until 1992 (US Dept. of Energy, 2000). The nuclear testing increased the levels of radiocarbon in the atmosphere by 100% and by 20% in the oceans. The increased levels of ^{14}C left a dated mark that is often referred to as the "bomb chronometer." The radiocarbon was incorporated into hermatypic corals in concentrations proportional to ambient levels in the water column (Druffel, 1980).

Kalish (1993) demonstrated that otoliths also incorporated ^{14}C in amounts proportional to the surrounding water column. Measurements of radiocarbon derived from seawater and corals provide a clear indication of radiocarbon levels at different points in time. The bomb-related increase in ocean radiocarbon can be used for age validation because of the discrete nature of the radiocarbon. The objective of this method is to select fish with presumed birth dates during the 1960-1970 increase in oceanic ^{14}C. Due to the dramatic increase of ^{14}C during this time period ages can be validated to within six months of the estimated birth date for fish born in the mid 1960's (Kalish,

1993). Comparisons of levels of radiocarbon found in the cores of otoliths to known levels found in corals can confirm the presumed birth dates of fish (Kalish, 1995). Analysis of bomb produced ^{14}C has provided successful age validation for Gulf of Mexico red snapper (Baker and Wilson, 2001) and other commercially important species around the world (Kalish, 1993; Campana, 1997; Campana and Jones, 1998).

The most common reproductive strategy of grouper species is protogynous hermaphroditism (Shapiro, 1987). Fish begin life as females, mature sexually and reproduce as females and eventually transform into males (Shapiro, 1987). Reproductive studies when combined with age and growth information allow for a detailed description of the biology of a species (Moe, 1969; Bullock et al., 1992; Crabtree and Bullock, 1998; Wyanski et al., 2000; Harris et al., 2002). Gonads (testes and ovaries) are collected to determine the sex of an individual and its current reproductive state. Reproductive research provides information regarding the sex ratio of the population, time and duration of the spawning season, spawning frequency, age and length of sexual maturity and in the case of hermaphrodites, age and length of sexual transition (Coleman et al., 1996; Collins et al., 1998; El-Sayed and Abdel-Bary, 1999; Rhodes and Sadovy, 2002; Brule et al., 2003).

Previous yellowedge grouper life history studies are limited to fish from South Carolina (Keener, 1984), the eastern GOM (Bullock et al., 1996) and Trinidad and Tobago (Manickchand-Heileman and Phillip, 2000). Limited age estimates were only determined for South Carolina and Trinidad and Tobago yellowedge grouper. Reproductive research was conducted on yellowedge grouper collected in the eastern GOM during 1977-1980 (Bullock et al., 1996). However, current reproductive research

is necessary to determine if fishing pressure has impacted the population structure of yellowedge grouper in the GOM. Fishing pressure may tend to decrease the relative abundance of males because males are larger in size and larger fish tend to be targeted first (Coleman et al., 1996). Reductions in age and size at sexual maturity or sexual transition are also biological responses observed as a result of fishing (Huntsman and Schaaf, 1994).

The objectives of this study were to provide the first available age estimates of yellowedge grouper in the northern GOM and validate observed age estimates using bomb-produced radiocarbon. Additionally, the current length-frequency and age-frequency distributions were compared to past distributions to evaluate the null hypothesis that no changes have occurred over time. Reproductive information such as current size and age at maturity, reproductive season and spawning frequency were determined. A comparison of the sex ratio of yellowedge grouper harvested today and fish harvested over 25 years ago was made in order to asses the null hypothesis that there has not been a shift in the sex ratio of the population. Finally, age and reproductive data results were used to make comparisons between the eastern and western GOM in order to evaluate if differences existed between the two regions. Life history research is significant because fisheries managers must be aware of biological information in order to design regulations that are appropriate to manage the fishery based on the biology of a species.

Pilot Study

My initial yellowedge grouper age and growth research began in August, 2000 with a pilot study entitled, "A preliminary investigation of sagittal otoliths as ageing

structures for yellowedge grouper, *Epinephelus flavolimbatus*". The pilot study was deemed necessary due to the difficulties previous investigators reported ageing this species. A data base was developed, all available otoliths (*n*=823) were cataloged and a small sample of otoliths (*n*=125) were sectioned for ageing (See Methodology below).

Numerous techniques were attempted to enhance otolith growth increments, including examining otoliths in clove oil and water, and using both transmitted and reflected light. In addition, histological stains were applied to mounted and unmounted sections. Neutral red and Toluidine blue, both with and without acetic acid, were applied for twenty minutes. The stains did not enhance existing growth increments, but rather only darkened the sections and made them more difficult to interpret. Otoliths were also sent to Old Dominion University for application of a new "baking technique" in an attempt to enhance the visibility of growth increments. The procedure involved baking otolith sections in a furnace at 400°C for a few seconds to minutes until a caramel color was achieved, but this method did not enhance the visibility of yellowedge grouper otolith growth increments.

Results of the study indicated that yellowedge grouper were difficult but not impossible to age. Growth increments were visible on the majority of the otoliths when viewed using transmitted light without enhancement. Consequently, I expanded my age and growth study of yellowedge grouper.

Methodology

Sample Acquisition

Yellowedge grouper (*n*=2,153) were collected from 1979-2005. However, sampling effort was not evenly distributed temporally, and several years were represented

by few or no samples (Table 1). The majority of samples (82.7%) were obtained from the National Marine Fisheries Service (NMFS), Panama City, Florida Laboratory archive collection with the remaining samples (17.1%) collected by the commercial fishery.

Table 1. Number of samples (O=otoliths, G=gonads) collected each year on National Marine Fisheries Service research cruises, by Trip Interview Program port samples and the commercial fishery. The percent sampled by year refers to the number of fish sampled per year because in most cases otoliths and gonads were collected from the same fish.

Year	Sample type	Frequency	Percent sampled by year	Cumulative percent
1979	O	8	0.4	0.4
1982	O	14	0.7	1.0
1983	O	31	1.4	2.5
1984	O	31	1.4	3.9
1985	O	12	0.6	4.5
1986	O	28	1.3	5.8
1987	O	7	0.3	6.1
1988	O	10	0.5	6.6
1989	O	6	0.3	6.9
1991	O	293	13.6	20.5
1992	O	74	4	23.9
1993	O	25	1.2	25.1
1994	O	2	0.1	25.2
1998	O	50	2.3	27.5
1999	O	100		
	G	42	4.7	32.2
2000	O	227		
	G	32	10.6	42.8
2001	O	701		
	G	32	32.6	75.3
2002	O	49		
	G	44	2.3	77.6
2003	O	254		
	G	244	11.8	89.5
2004	O	162		
	G	146	7.5	97.0
2005	O	65		
	G	65	3.0	100.0
Total	O	2153		
	G	605		

The NMFS archive collection was comprised of 1,781 otoliths collected from 1979-2005 primarily by Trip Interview Program (TIP) port samplers (79.1%) and from NMFS, Pascagoula, Mississippi Laboratory (MS Lab) scientific cruises (20.9%). A total of 605 reproductive samples (gonads) were collected from 1999-2005. Gonads were collected from the commercial fishery (*n*=356), the MS Lab (*n*=240) and TIP agents (*n*=9). The TIP is a sampling program conducted by Federal and state port agents. A dockside interview was conducted with the captain/crew regarding the individual fishing trip. Agents target the commercial, charter boat and head boat fishing sectors, however, since few samples were collected from the charter boat (*n*=27) and head boat (*n*=53) sectors all TIP data was grouped as fishery dependent data for statistical analysis. The primary focus of the data is to obtain size frequency information for species involved in Federal Fishery Management Plans (FMP). Information on the gear used, area and depth fished, fishing effort and both length (total length (TL) or fork length (FL) and weight (whole or gutted) of the catch is recorded during the interview. Otoliths (primarily the left) and gonads (when available) were removed from species of interest. The MS Lab conducted annual longline surveys in depths from 9-366 m. Sampling protocol followed the same procedures as the commercial sampling protocol except all fish captured were sampled, both otoliths were removed and more detailed environmental data were collected. All removed otoliths were stored dry in labeled envelopes and gonads were stored in 10% neutral buffered formalin (NBF).

In order to increase the number of samples, a Cooperative Research Program grant was obtained to hire a commercial fisherman to sample yellowedge grouper. Yellowedge grouper were collected, from the eastern GOM, using commercial bottom

longline gear on a monthly basis for nineteen months from April 2003 through October 2004. A maximum of sixty fish of varying sizes (30 fish < 650 mm TL and 30 fish > 650 mm TL) were to be collected during each trip. Location of capture, water depth, bottom topography, number of hooks used, soak time and time of capture were recorded. Weight (kg) and length (TL and fork (FL), (mm)) of each fish were also recorded and otoliths and gonads were removed (within 12 hours of capture) from each fish. Weather, mechanical trouble, vessel issues and closure of the commercial fishery from July 15, 2004 through December 31, 2004 prohibited the sampling of yellowedge grouper on a monthly basis. Samples were only collected on ten fishing trips and the sampling period was extended through March 2005 to allow for the collection of additional samples.

A database was developed that included specimen information for length, weight, date of capture, depth and location (latitude/longitude or state). All lengths reported in this study are TL. The Gulf of Mexico (GOM) was divided into the eastern GOM and western GOM at the 89° 15' W longitude for comparison between the two regions and into three Zones, eastern (E), central (C) and western (W), based on NMFS Faunal Zones (National Marine Fisheries Service, 2001). Because FL was often recorded by TIP port agents, a linear regression (TL=1.069*FL-16.712; n=1,008; R^2=0.997) was used to convert values of FL to TL.

Age and Growth

A total of 2,152 otoliths were collected, however, the majority (74.8%) were collected from 1998-2005 because of an increase in effort by TIP agents in 2000 and 2001 resulting in the collection of 853 otoliths. Due to the large number of otoliths collected, not all TIP otoliths were processed and aged. Random numbers were

generated using Microsoft Excel (Microsoft Corp., Redmond, WA) and collection numbers were selected to reduce the number of otoliths selected for ageing by approximately 50%. However, all otoliths from fish ≤ 450 mm TL or ≥ 950 mm TL were aged to adequately represent the extreme ends of the size distribution. All otoliths from 1993 (*n*=26), 1994 (*n*=2) and 1998 (*n*=50) were omitted regardless of fish size because sample sizes were too small to adequately represent the age structure for those years. Omitted otoliths comprised only 6.9% and 3.1% of otoliths collected during the 1991-1994 and 1998-2005 time periods, respectively. All otoliths collected in 2002-2005 from either scientific surveys or the commercial fishery and were aged. All sagittal otoliths were weighed (g), processed and analyzed following traditional ageing procedures.

Accelerator Mass Spectrometry (AMS). A Marine Fisheries Initiative Program (MARFIN) grant was obtained to fund the age validation procedure. An age validation technique was first applied to validate annual growth increment formation and confirm observed ages prior to commencement of traditional age and growth research. This procedure analyzed the levels of $\Delta^{14}C$ within the core of the otolith, which was then used to validate the age of the fish. Sagittal otoliths used for age validation were selected from the NMFS, Panama City, Florida Laboratory otolith archives. Otoliths were collected from the commercial fishing industry and during scientific research studies. In order to validate ages throughout the life history, selected yellowedge grouper (*n*=51) ranged from 177-1,160 mm TL. Fish were selected based on size strata or the availability of both otoliths. Otoliths were selected based on the estimated birth date determined from annual growth increment counts; only otoliths with discernable marks were included. Fish

selected had a wide range of presumed birth dates (1915-1999) and ranged in estimated age from 1-85 years old.

The right otolith was primarily used for ^{14}C core analysis. The otolith was embedded in epoxy resin and two to three sections (0.7 mm width) were removed from each selected otolith, which typically provided adequate sample material for the removal of the core region. In order to obtain enough material for otolith core analysis (minimum of 3.0 mg for a standard sample), core sections and often as much as the first two years of growth were extracted from otoliths. Desired sections were first identified using a Staedtler 005-005 pigment liner pen (Baker and Wilson, 2001). Cores (n=11) from the first batch of samples submitted were removed by a dentist who used a standard dental drill to isolate the core. Remaining core sections were extracted using a Dremel Multipro rotary tool fitted with a 1.4 mm diamond needle bit. The Dremel powdered the otolith which allowed for homogeneity of duplicate samples. First the distal portion below the core of the otolith was removed using the Dremel to avoid contamination by other growth increments. The drill bit was replaced with a clean bit for use with each otolith. Cores were stored in sterile vials which were first rinsed three times each with 1 N HCl followed by distilled water and then dried in an oven. Blind duplicate samples of nine otolith cores were also submitted for ^{14}C analysis to determine the accuracy of our methodology and test the precision of the analysis. A paired-samples t-test was used to evaluate if significant differences existed between core samples and corresponding blind duplicates.

In order to validate ages of fish born prior to 1960 and thus prior to the oceanic increase in ^{14}C, a new technique which isolated multiple areas (besides the core) on a

single otolith was applied. Areas were isolated using the National Ocean Sciences Foundation digital microsampler. The objective was to isolate regions on the otolith, determined by counting growth increments, which corresponded to a time period either prior to the nuclear bomb testing (pre-bomb) or after the 1960's increase in ^{14}C (post-bomb). If results matched the proper time period assigned it would indicate how much growth occurred prior to the nuclear bomb testing and also indicate that growth increments were deposited annually. Fish (n=8) selected had estimated ages, obtained by counting otoliths growth increments, between 21-85 years and ranged in size from 755 to 1,148 mm TL. Each otolith was embedded in epoxy resin and a thin transverse section (1.1 mm) was taken. A photograph of the otolith section was taken using a Nikon SMZ-1500 Stereomicroscope fitted with a Nikon DMX-1200 digital camera and used to create digital points which the microsampler used as a reference. The otolith section was adhered to the base plate of the microsampler using Crystal Bond. Areas on the otolith to be isolated and removed were digitized using the microsampler. Growth increments were isolated from the sulcus toward the ventral side of the otolith. Since the amount of calcium carbonate deposited decreases with age, isolated otolith sections contained numerous years of growth (~2-12 years) to obtain enough material for ^{14}C analysis. The microsampler used a drill to shave the isolated sections off the otolith. Sections removed were nearly as long as the ventral section of the otolith and approximately 0.7 mm deep. Shavings were collected and stored in sterile glass vials until AMS processing. Many of the otolith areas isolated were formed during the 1960-1973 increase in ^{14}C.

The edge of one of the otoliths (sample #1424) collected in 2001 was also isolated to compare ^{14}C results with that of a one year old fish collected in 2000 to determine if

^{14}C levels were similar if isolated zones were formed in the same year. The edge included two years of growth (2000 and 2001). Yellowedge grouper adults are found in different areas and depths than juveniles; isolated section analysis will only provide comparable results if otoliths absorb ^{14}C at the same rate throughout the lifespan of the fish. Otolith samples were submitted to the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) Facility at the Woods Hole Oceanographic Institute (WHOI) for analysis. Samples were submitted in seven separate batches over the course of 2.5 years. At the NOSAMS facility the otolith cores (n=48) were converted into pure carbon in the form of graphite and analyzed for radiocarbon using the procedures described by Baker and Wilson (2001). Samples were also analyzed for ^{13}C, which was used to correct for natural and machine fractionation effects. Radiocarbon values are reported as $\Delta^{14}C$, which is the per mil (‰) deviation of the sample from the radiocarbon activity of 19^{th} century wood, after corrections for isotopic fractionation and sample age decay prior to 1950 AD (Stuiver and Polach, 1977).

Radiocarbon results were compared to results from published $\Delta^{14}C$ values from GOM red snapper (Baker and Wilson, 2001) and corals collected in Belize, Bermuda and Islamorada, Florida (Druffel, 1989; Druffel, 1980; Druffel, personal communication) to determine if yellowedge grouper ^{14}C values followed the same trend as other fish and corals from a similar region. An analysis of covariance (ANCOVA) test for homogeneity of slopes was used to determine if $\Delta^{14}C$ values from 1960-1973 for yellowedge grouper, red snapper and coral were significantly different. If yellowedge grouper results were similar they would support the ages obtained from counting otolith growth increments.

Traditional Ageing Procedure. Two different methods were used to mount otoliths (*n*=1,687) depending on the size of the otolith. Small otoliths were embedded whole in an epoxy resin. A Buehler Isomet Low Speed saw with a diamond dicing blade was used to cut a transverse section (0.6 mm) through the primordium (core). Sections were polished with 1500-grit fine grade silicon carbide paper and mounted with Crystal Bond to a glass slide**.** Final polishing was completed using a Foredom Bench polisher and Buehler 0.3 micron polishing compound. Larger otoliths (> 0.09 g) were mounted to a glass slide using Lakeside Thermoplasic Cement. Otoliths were sectioned (0.6 mm) using a Buehler Isomet saw and sections adhered to a second slide using Cytoseal Mounting Medium.

The otolith sections were examined using a dissecting microscope with transmitted light at a magnification of 10x to 50x. The number of opaque growth increments was counted 3-5 times per fish and the median age was recorded. Each otolith was independently aged by two readers who lacked knowledge of specimen length or date of capture. Due to difficulty experienced by previous investigators otoliths were assigned a readability (Table 2) based on Kuo and Tanaka (1984). Otoliths with reader age differences ≤2 years were assigned my age estimate since I was the primary and more experienced reader. Fish ages with reader age differences ≥3 years were reviewed again by both readers. If readers could not agree upon an age the sample was excluded from the study. Indices of reader precision were determined using percent agreement (*e.g.* ±1 or ±2 growth increments), average percent error (APE) and coefficient of variation (CV; Beamish and Fournier, 1981; Chang, 1982).

Table 2. 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).

Otolith Weight Comparisons. A paired samples *t*-test was used to determine if there was a significant difference between the weight of the left and right otoliths since left otoliths were primarily collected. Linear regression was used to determine if a relationship existed between fish age and otolith weight.

Age-Length Structure. In order to determine general biological parameters all age data were combined regardless of sector (fishery independent, i.e. NMFS, or dependent), gear, location or year to illustrate yellowedge grouper size-at-age. Age-length frequencies were produced by sector, gear, year of capture (time period): 1) 1979-1989, 2) 1991-1992 and 3) 1999-2005) and Zone. The lack of samples collected during several years resulted in time periods without an equal number of years in each period and gaps between periods. A Mann-Whitney *U* test was used to determine if there was a significant difference between ages and total lengths of yellowedge grouper collected by fishery dependent verses independent sectors. A Kruskal-Wallis test was used to

determine if there was a significant difference between ages and total lengths of yellowedge grouper collected by gear type (bottom longline, hand line and all other gears combined). The outcome of statistical analyses dictated if and how samples were combined for additional analyses. A Kruskal-Wallis test was used to test the null hypothesis that there was no difference in the median ages of yellowedge grouper captured between each time period. Yellowedge grouper sampled during each time period were evaluated using a Kolmogorov-Smirnov two-sample test (K-S) to determine if there has been a significant change in length-frequency between each selected time period. The test was run three times to allow for all comparisons. Median size and age of yellowedge grouper collected during 1999-2005 in the eastern and western GOM were compared using a Mann-Whitney *U* test to test the null hypothesis that there was no difference between the two regions.

*von Bertalanffy growth curve*s. Observed lengths at age were used to construct the von Bertalanffy (1938) theoretical growth model which is described as:

$$L_t = L_\infty (1 - e^{-K(t-t_0)})$$

where:

L_∞ = theoretical maximum length;

K = growth rate coefficient;

t = age in years;

t_0 = theoretical age at length zero and;

e = exponent for natural logarithms.

The von Bertalanffy growth model was constructed with all aged yellowedge grouper combined, for each time period and the eastern verses western GOM (using only 1999-

2005 fish). Model parameters were estimated using least-squares non-linear regression. Growth curves and parameters (L_∞, K, t_0) were compared between time periods using likelihood ratio tests (LRT) (Haddon, 2001) to determine if there had been a change in growth over time. The LRT tests the hypothesis that the curves are independent (referred to as the base case) verses the alternate hypothesis that the curves are samples from a single population (coincident curve) (Haddon, 2001). If the test of coincident curves was significant multiple pair-wise comparisons were used to determine which curves and parameters were significant. Parameters from the von Bertalanffy growth equation were subsequently used in the yield-per-recruit and spawning stock biomass-per-recruit models and to estimate natural mortality.

Statistical analysis was conducted using SPSS 14.0 (SPSS, Inc., Chicago, IL) or Microsoft Excel (Microsoft Corp., Redmond, WA). All data were tested for normality and homogeneity of variance. If data were not normally distributed a natural log transformation was used to normalize the data. Nonparametric tests were conducted if there were large violations of these assumptions. Results were considered significant if $P < 0.05$.

Reproduction

Gonad Processing. Whole gonad weights were from fixed gonads, therefore, the change in weight due to preservation was determined. Yellowedge grouper gonads collected on the MS Lab 2004 longline cruise were weighed fresh (g) and then preserved in 10% NBF. Gonads were weighed approximately every 30 days until the there was no change in weight from the previous month. A paired samples *t*-test was used to determine that there was a significant difference in the weight of fresh and preserved

gonads. The fixed gonad weights were adjusted to represent fresh weights using linear regression correction factors based on histological stage.

All fixed gonads were weighed to the nearest 0.01 g. A small section (~1 cm^3) was removed, primarily from the posterior end of the gonad, from each sampled fish and placed in a cassette for histological processing. To determine whether the sub-sample represented homogeneous oocyte development throughout the ovary three sections (anterior, center, and posterior) from each lobe (right, left) of the ovary of 10 fish visually classified in the late maturation class were removed and examined for developmental stage. Three random sites on each histological section were viewed at 10x magnification using a compound microscope fitted with a 10 x 10 mm, 1 mm square, reticle grid. The number and percent of all cortical alveoli, vitellogenic (yolk granule and yolk globule) and final oocyte maturation (FOM) oocytes was determined. Percents were arcsine square root transformed prior to analysis. A linear mixed model with fixed effects was used to determine if the mean of each oocyte type differed throughout the gonad. Fixed effects included in the model were the intercept, lobe and section. Fish was specified as a subject variable, lobe and section were repeated variables and compound symmetry was specified as the covariance structure of the residuals. The analysis was conducted for each of the three oocyte types. Homogeneous oocyte development would validate the use of a single sub-sample for each fish.

Initial histological processing occurred at the University of Southern Mississippi's Gulf Coast Research Laboratory, Ocean Springs, MS, but subsequent samples were sent to the Louisiana State University Histopathology Laboratory in Baton Rouge, LA for histological processing. Cassettes were rinsed in fresh water overnight then soaked for

two hours each in 60% EtOH, 70% EtOH and 70% EtOH. Cassettes were placed in a Shandon Hypercenter II for dehydration, clearing and paraffin infusion (Table 3). Tissues were embedded in Paraplast and sectioned using an American Optical rotary microtome to a thickness of 4 μm and mounted on glass slides. Sections were stained with hematoxylin-1 and eosin-Y following standard histological procedures (Table 4) and cover slipped with Shandon Mounting Medium.

Histological slides were viewed using a compound microscope at 40 to 400x magnification to determine reproductive class. Gonads were classified using maturity classes developed by Sadovy and Shapiro (1987), Grier and Taylor (1998) and Brown-Peterson (2003) (Table 5). Females were considered sexually mature if cortical alveoli oocytes or more advanced oocyte stages were present.

Table 3. Shandon Hypercenter II tissue processing. Tissue remains in each step for one hour.

Solution	**Setup Procedure**
1.) 70% EtOH	Discard
2.) 80% EtOH	Discard
3.) 95% EtOH	Discard
4.) 95% EtOH	Rotate to 3
5.) 100% EtOH	Discard
6.) 100% EtOH	Rotate to 5
7.) 100% EtOH	Rotate to 6
8.) Xylene sub	Discard
9.) Xylene sub	Rotate to 8
10.) Xylene sub	Rotate to 9
11.) Paraplast	Discard
12.) Paraplast	Rotate to 11

Table 4. Staining procedures used to make yellowedge grouper histological slides.

Station	Solution	Procedure
1	Xylene Substitute	Soak 3 minutes
2	Xylene Substitute	Soak 3 minutes
3	Xylene Substitute	Soak 3 minutes
4	100% EtOH	10 dips
5	100% EtOH	10 dips
6	95% EtOH	10 dips
7	95% EtOH	10 dips
8	80% EtOH	10 dips
9	80% EtOH	10 dips
10	50% EtOH	10 dips
11	Distilled water	Soak 1 minute
12	Hematoxylin	Soak 3-5 minutes
13	Water	Rinse well
14	Acid water	2 dips
15	Water	Rinse well
16	Blueing water	Soak 30 seconds
17	Water	Rinse well
18	95% EtOH	Rinse well
19	Eosin	Soak 45 sec. - 1.5 minutes
20		Blot slide
21	95% EtOH	10 dips
22	95% EtOH	10 dips
23	95% EtOH	10 dips
24	100% EtOH	Soak 1 minute
25	100% EtOH	Soak 1 minute
26	100% EtOH	Soak 1 minute
27	Xylene Substitute	Soak 1 minute
28	Xylene Substitute	Soak 1 minute
29	Xylene Substitute	Soak 1 minute
30	Xylene Substitute	Soak 1 minute

Table 5. Yellowedge grouper sexual maturation classes. Classes developed by Sadovy and Shapiro (1987), Grier and Taylor (1998) and Brown-Peterson (2003)

Sex	Maturation Class	Acronym	Description
Female	Immature	I	Only primary growth oocytes present
Female	Early maturation	EM	Cortical alveoli oocytes present; small lipids in cytoplasm
Female	Mid-maturation	MM	Yolk granule oocytes predominant; numerous cortical alveoli and yolk globule oocytes present
Female	Late maturation	LM	Fully grown yolk globule oocytes predominant; all other stages present; post-ovulatory follicles may be present
Female	Final oocyte maturation	FOM	Hydrated oocytes, oocytes with advanced lipid and/or yolk coalescence or migratory nucleus present; all other stages present
Female	Regressing	RGS	Majority of oocytes experiencing some level of atresia; all stages may be present in limited numbers
Female	Regenerating	RGT	Only primary growth oocytes present; some late atretic oocytes; evidence of prior spawning
	Transitional	T	Spermatocytes, spermatids or spermatozoa present AND degenerating ovarian tissue observed; atretic follicles and oocytes in early stages of atresia
Male	Early maturation	EM	Spermatogonia found throughout testis; spermatozoa can be in ducts; spermatocysts line lobule walls as continuous germinal epithelium throughout
Male	Mid-maturation	MM	Secondary spermatogonia present; few primary spermatogonia; spermatozoa observed in ducts; discontinuous germinal epithelium at termini of lobules only
Male	Late maturation	LM	Presence of discontinuous germinal epithelium near testis ducts; spermatocysts contain spermatocytes, spermatids or spermatozoa; spermatozoa found in lumina of lobes and ducts
Male	Regressing	RGS	Decreased number of spermatocysts containing spermatids and spermatozoa; extension of a sperm-filled lobular lumen
Male	Regenerating	RGT	Primary spermatogonia; continuous germinal epithelium; sometimes residual spermatozoa in lumen

Reproductive Analysis

Size and age at maturity. 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 with all females included and also on only active females to allow for comparison with Bullock et al. (1996). Active females were classified as those with vitellogenic oocytes present. The analyses was also run for yellowedge grouper collected in the eastern and western GOM to determine if differences existed between regions and for additional comparison against Bullock et al. (1996) since their samples were from the eastern GOM only.

Size and age at sexual transition. 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 in the population had transformed into males. The analysis was completed using all data combined and also for the eastern and western GOM. All females were included in the analysis.

Sex Ratio. Sex ratio (male:female) was determined by dividing the number of females by the number of males for all yellowedge grouper combined and for the eastern GOM and western GOM. A chi-square test was used to determine if the ratio differed significantly from 1:1 and if there was a significant difference between the sex ratio of yellowedge grouper collected in the eastern GOM and the western GOM. A chi-square test was also used to determine if there was a significant difference between the sex ratio of yellowedge grouper sampled from 1977-1980 (Bullock et al., 1996) and fish sampled during 1999-2005. In addition, a chi-square test was used to determine if there was a significant difference between the sex ratio of 1:1.8 (Bullock et al., 1996) and yellowedge

grouper collected during the current study from the eastern GOM allowing for a more direct comparison between the two time periods.

Spawning Season. The gonadosomatic index (*GSI*) was calculated for males and females using the following formula:

$$GSI = (GW/(TW\text{-}GW)) * 100;$$

where *GW* = total fresh gonad weight (g) and *TW* = total fish weight (g). The relationship between GSI and gonad-free body weight was tested using linear regression. If no significant relationship was observed, GSI could be used as an indicator of reproductive readiness. Monthly median GSI values were also plotted to show seasonal reproductive patterns. A One-way analysis of variance (ANOVA) was used to examine if monthly differences in mean GSI values existed. Monthly percentages of each maturation class were determined to see how closely ovarian maturation and monthly mean GSI values overlapped. All GSI values were arcsine-square root transformed prior to statistical analysis.

Spawning frequency. Monthly spawning frequency (the number of days between each spawning event) and annual spawning frequency (the number of times a female spawns each year) were determined using techniques developed by Hunter and Macewicz (1985) and Brown-Peterson and Warren (2001). The percentages of spawning-capable females with ovaries containing oocytes in 1) FOM and 2) 0-24 hour postovulatory follicles (POF) were calculated on a monthly basis (*o*). Yellowedge grouper were considered spawning-capable if oocytes were in the late maturation or FOM stage. Monthly spawning frequency (*d*) was determined by dividing 100% by *o*. Annual spawning frequency was determined by dividing the duration of the spawning season by

d. The duration of the spawning season was calculated by summing the days of the months in which females with oocytes in FOM were collected. A chi-squared test was used to determine if spawning frequency varied on a monthly basis for each method.

Batch, relative and annual fecundity. Fecundity analysis was not conducted because all of the gonads collected were lost in Hurricane Katrina prior to analysis. Several attempts were made to obtain additional samples, however, none of the yellowedge grouper collected were suitable for fecundity analysis.

The relationships between gonad weight to total length, whole weight and age were used as a proxy for fecundity. Only females with vitellogenic or hydrated oocytes collected during the peak spawning months of July, August and September were used. Females classified as regressing were also excluded from the analysis.

Statistical analysis was conducted using SPSS 14.0 (SPSS, Inc., Chicago, IL) or SigmaStat 3.5 (Systat Software Inc., Point Richmond, CA). All data were tested for normality and homogeneity of variance. If data were not normally distributed a natural log transformation was used to normalize the data. Nonparametric tests were conducted if there were large violations of these assumptions. Results were considered significant if $P < 0.05$.

Results

Age validation

Analysis of otolith cores produced a $\Delta^{14}C$ curve for yellowedge grouper when the radiocarbon values were plotted against the estimated ages obtained from counting annual growth increments on the otoliths (Figure 1). For example, fish #1425, a 31 year old yellowedge grouper collected in 2001, had an estimated birth date of 1970 and a

corresponding $\Delta^{14}C$ value of 136.3‰.

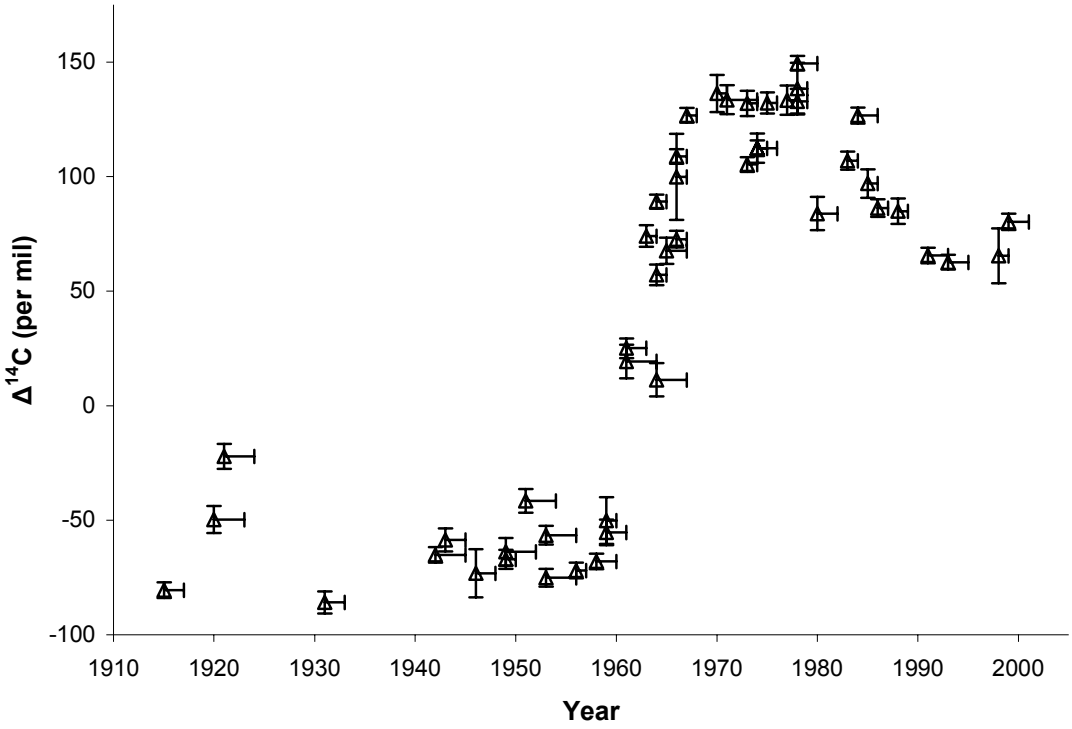


Figure 1. Values of $\Delta^{14}C$ (±1 SD) from otolith core analysis plotted against birth date. Birth dates determined by counting annual growth increments from sagittal otoliths. The x-axis error bars represent number of years in the core sample, triangles represent the core.

Levels of $\Delta^{14}C$ from fish with presumed birth dates prior to the nuclear bomb testing had negative core $\Delta^{14}C$ levels ranging from -85.9 to -22.1‰ (Table 6). Yellowedge grouper born after 1960 had elevated core $\Delta^{14}C$ levels ranging from 11.3 to 149.4‰ with the highest $\Delta^{14}C$ level recorded in 1978. A significant linear relationship existed for yellowedge grouper born from 1960-1978 (n=22, p=0.000, R^2=0.680). Fish born after 1978 (n=10) had gradually decreasing core levels of $\Delta^{14}C$ ranging from

Table 6. Yellowedge grouper data and core sample radiocarbon results. Age and birth year were determined by counting otolith annual growth increments. Year(s) sampled identifies core sample year and additional consecutive years, if any, that were submitted for ^{14}C analysis. $\delta^{13}C$ ($^{13}C/^{12}C$) was used to correct for isotopic fractionation to calculate $\Delta^{14}C$ and SD refers to standard deviation of the $\Delta^{14}C$ result.

Sample ID	Fork Length (mm)	Collection year	Age (years)	Birth year	Otolith weight (mg)	Sample weight (mg)	Year(s) sampled	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	± 1 SD (‰)
753	1090	2000	85	1915	6991	6.70	1915-1916	-3.90	-80.5	3.4
206	1090	1991	71	1920	4663	21.61	1920-1922	-2.98	-49.7	5.9
333	1030	1991	70	1921	4566	24.07	1921-1923	-3.96	-22.1	5.4
1424	888	2001	70	1931	3261	3.40	1931-1932	-4.60	-85.9	4.9
415	1020	1992	50	1942	3731	5.20	1942-1944	-3.60	-65.1	3.4
283	1030	1991	48	1943	2969	4.20	1943-1945	-4.39	-58.6	5.1
1097	920	2001	55	1946	2713	3.70	1946-1947	-1.29	-73.2	10.5
271	810	1991	42	1949	1594	22.11	1949-1951	-3.59	-63.8	6.0
1457	867	1979	30	1949	2377	7.40	1949	-4.44	-67.1	4.2
329	970	1991	40	1951	2652	16.72	1951-1953	-4.11	-41.6	5.2
253	930	1991	38	1953	2895	26.80	1953-1955	-3.56	-56.6	4.1
325	1000	1991	38	1953	2703	21.82	1953-1955	-3.74	-75.1	3.9
1577	900	2001	45	1956	2497	8.50	1956	-4.37	-71.9	3.4
1486	590	1983	25	1958	937	4.10	1958-1959	-5.24	-68.0	3.4
1487	620	1983	24	1959	910	3.10	1959	-5.62	-50.2	10.2
1578	950	2001	42	1959	2553	6.10	1959-1960	-4.83	-55.3	5.6
197	870	1991	30	1961	2359	19.76	1961-1963	-4.43	19.3	7.3
1466	708	1984	23	1961	1332	4.50	1961-1962	-4.28	25.0	4.2
1473	708	1984	21	1963	1068	4.90	1963	-4.34	74.0	4.7
372	1044	1992	28	1964	2320	18.05	1964-1966	-3.72	11.3	7.3
1138	965	2001	37	1964	3049	3.20	1964	-4.78	57.1	4.5
1521	643	1984	20	1964	1016	6.20	1964	-4.77	89.0	3.1
1502	625	1983	18	1965	938	5.90	1965-1966	-5.31	67.5	5.7
1465	760	1982	16	1966	1282	6.00	1966	-3.70	72.6	3.6
1507	505	1983	17	1966	831	5.40	1966	-5.00	108.8	3.2
1520	665	1984	18	1966	949	6.40	1966	-4.64	99.8	18.8
1483	630	1984	17	1967	859	6.00	1967	-5.01	126.7	3.2
1425	940	2001	31	1970	2507	6.30	1970	-4.51	136.3	8.1
649	985	2000	29	1971	2460	28.90	1971-1973	-4.20	133.5	6.3
1442	792	2001	28	1973	1722	5.40	1973	-5.00	105.2	3.2
1482	596	1984	11	1973	637	3.80	1973	-5.41	131.9	5.5
1434	807	2001	27	1974	1819	9.80	1974-1975	-5.06	112.3	6.4
1471	632	1984	10	1974	710	6.40	1974	-4.52	112.3	3.4
516	945	1999	24	1975	2388	9.20	1975	-4.50	132.1	4.6
1469	563	1984	7	1977	607	5.70	1977	-5.54	133.3	6.4
631	750	2000	22	1978	1429	10.15	1978-1979	-4.95	149.4	3.2
640	765	2000	22	1978	1650	6.20	1987	-5.05	138.4	11.3
1504	474	1983	5	1978	432	5.70	1978	-4.77	132.8	5.4
1423	842	2001	21	1980	1725	10.00	1980-1981	-3.67	83.8	7.3
648	759	2000	17	1983	1411	8.69	1983	-5.00	106.9	4.0
634	715	2000	16	1984	1103	11.00	1984-1985	-5.00	126.6	3.5
650	669	2000	15	1985	1140	7.40	1985	-5.29	96.9	6.2
1441	743	2001	15	1986	1178	4.90	1986	-4.67	86.2	3.8
639	702	2000	12	1988	1098	6.50	1988	-4.75	84.8	5.6
636	565	2000	9	1991	694	5.48	1991-1992	-5.33	65.5	3.4
1437	550	2001	8	1993	571	8.00	1993-1994	-5.63	62.5	3.2
674	262	2000	2	1998	114	2.36	1998	-5.60	65.4	12.0
825	177	2000	1	1999	52	29.29	1999-2000	-6.21	80.2	3.5

126.5‰ to 62.5‰. Yellowedge grouper $\Delta^{14}C$ values declined at a rate almost three times slower than the initial increase and have yet to reach pre-bomb levels. An ANCOVA test for homogeneity of slopes indicated no significant difference (*df*=4, MS=724.6, *F*=1.056, *p*=0.386) between yellowedge grouper, red snapper and coral $\Delta^{14}C$ chronologies from 1960-1973 (Figure 2), the period of greatest increase in ^{14}C levels. These findings indicate that yellowedge grouper deposit a single annual growth increment.

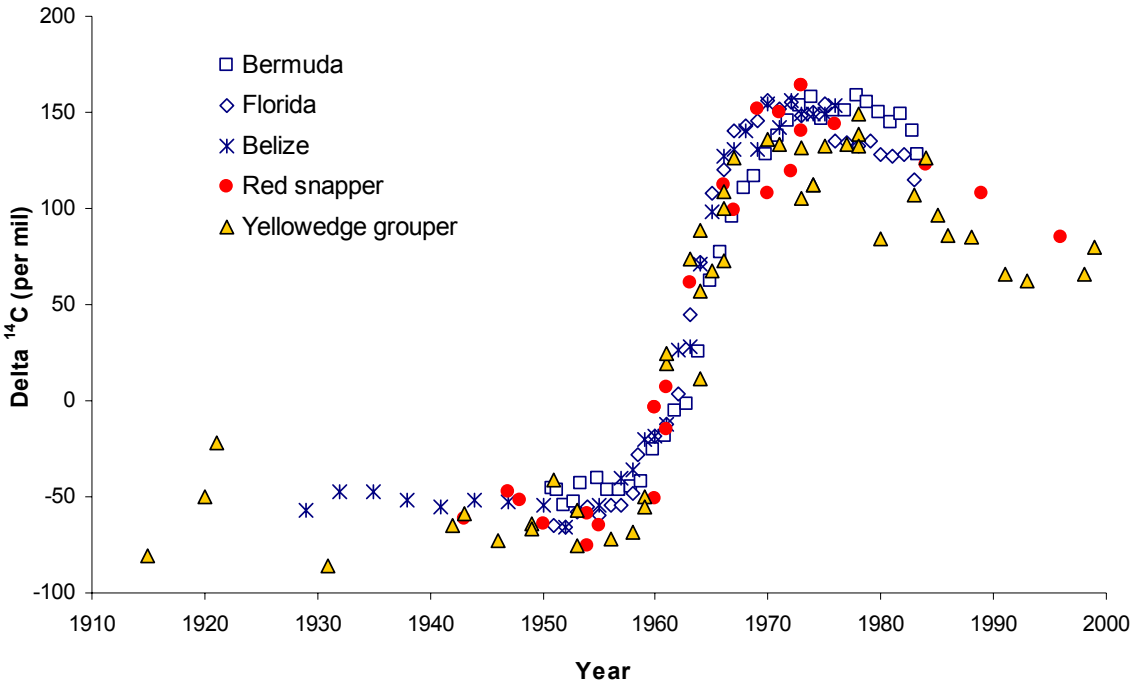


Figure 2. Values of $\Delta^{14}C$ from otolith and coral analyses plotted against birth/formation date. $\Delta^{14}C$ values are from yellowedge grouper otolith cores (*n*=48) and published $\Delta^{14}C$ chronologies for Gulf of Mexico red snapper (*n*=26) (Baker and Wilson, 2001) and corals from south Florida (*n*=33) (Druffel, 1989), Bermuda (*n*=35) (Druffel, 1989), and Belize (*n*=30) (Druffel, 1980). Yellowedge grouper data points represent year of birth although core samples may contain up to three years of growth.

Results of the blind duplicate samples indicated that the majority of blind duplicates and their subsequent core samples had similar levels of $\Delta^{14}C$ (Table 7). The absolute value of the difference between the core sample and blind duplicate ranged from 2.1 to 28.4 ‰. A paired-samples *t*-test indicated no significant difference between the samples (t_8=0.836, P=0.427) which supported the methodology used for core extraction and the precision of the ^{14}C analysis.

The isolated area analysis was used to validate the age of yellowedge grouper that were born prior to the 1960's increase in ^{14}C (Table 8). Figure 3 depicts a yellowedge grouper otolith from a fish that is believed to be 85 years old based on growth increment counts. Radiocarbon results from the first three isolated sections (ΔC^{14}=-80.5‰, -71.1‰, -94.7‰, respectively) indicated pre-bomb levels of ΔC^{14}. Each of those sections contained growth increments that were formed before 1960 since $\Delta^{14}C$ levels were <0‰ prior to the nuclear-bomb produced ^{14}C increase. The post-bomb isolated section had a positive ΔC^{14} value of 38.9‰ which indicated formation after 1960. Thus many of the growth increments contained in the section had to be deposited after 1960 in order to produce a positive $\Delta^{14}C$ value. The majority of isolated section results followed this same pattern; pre-bomb sections with negative $\Delta^{14}C$ values were discriminated from post-bomb sections with positive $\Delta^{14}C$ values. Results from the isolated otolith edge (85.9‰) were similar to that of the one year old (80.2‰) indicting that ^{14}C absorption is fairly constant throughout the life of the fish and that ^{14}C levels in juvenile and adult habitats are comparable.

Table 7. Blind duplicates of core samples. Blind duplicate samples were used to test reproducibility of the accelerator mass spectrometer instrument and methodology. Type represents core (C) or blind duplicate (D). Batch refers to which batch the samples were submitted in, both were submitted in the same batch.

Sample ID	Type	Batch	Year Born	$\Delta^{14}C$ (‰)	±1 SD (‰)	Core - Duplicate (‰)
325	C	2	1953	-75.1	3.9	-15.5
325	D		1953	-59.6	3.4	
329	C	1	1951	-41.6	5.2	23.2
329	D		1951	-64.8	3.8	
649	C	2	1971	133.5	6.3	-12.7
649	D		1971	146.2	4.4	
753	C	3	1915	-80.5	3.4	-1.8
753	D		1915	-78.7	5.2	
825	C	2	1999	80.2	3.5	-2.7
825	D		1999	82.9	3.3	
1097	C	4	1946	-73.2	10.5	9.3
1097	D		1946	-82.5	3.1	
1138	C	4	1964	57.1	4.5	11.8
1138	D		1964	45.3	3.7	
1425	C	5	1970	136.3	8.1	28.4
1425	D		1970	107.9	3.9	
1578	C	4	1959	-55.3	5.6	-2.1
1578	D		1959	-53.2	5	

Table 8. Results of yellowedge grouper isolated section analysis. Pre-bomb refers to otolith growth increments that were formed prior to the 1960's increase of ^{14}C. Post-bomb refers to otolith growth increments that were formed after the 1960's. Each isolated section contained approximately 2-12 years of growth increments.

Sample ID	Sample Description	Fish Age (Years)	Year Born	Sample Weight (mg)	$\Delta^{14}C$ (‰)	±1 SD (‰)
101 Z	Post-bomb	70	1921	4.1	-37.1	3.8
283 Z	Post-bomb	48	1943	2.8	-39.9	3.6
415 A	Pre-bomb	50	1942	4.0	-51.9	3.5
753 Z	Pre-bomb	85	1915	3.3	-71.1	7.5
753 Y	Pre-bomb	85	1915	2.9	-94.7	4.5
753 U	Post-bomb	85	1915	3.5	38.9	4.3
922 A	Pre-bomb	70	1931	3.3	-51.5	3.9
922 Z	Post-bomb	70	1931	4.4	45.2	3.8
1097 Z	Pre-bomb	55	1946	4.3	-74.7	3.9
1424 Z	Pre-bomb	70	1931	2.9	-101.3	3.3
1424 E	Post-bomb	70	1931	1.6	85.9	8.4
1470 Z	Post-bomb	21	1963	3.2	23.8	3.7
1470 A	Post-bomb	21	1963	3.4	30.9	4.0

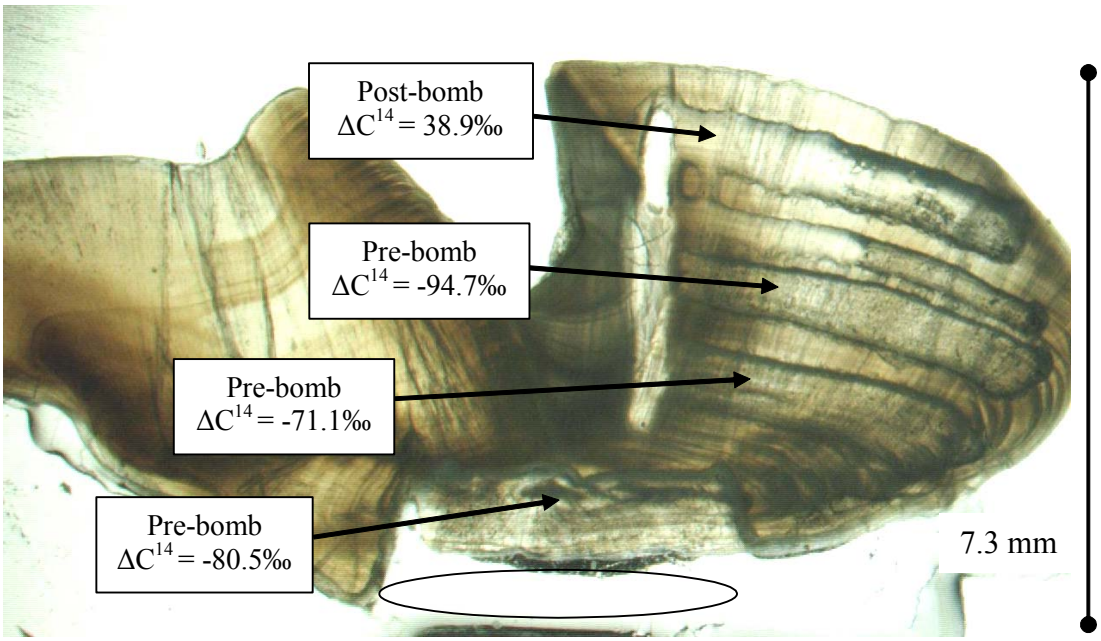


Figure 3. Otolith 753 with multiple growth increments removed. In order to obtain sufficient material, several growth increments (2-12 years of growth) were combined for ^{14}C analysis. Reported $\Delta^{14}C$ values represent a combination of years instead of a single point estimate. The circled area represents the distal area below the core which was removed prior to sampling to avoid contamination by other growth increments.

Age and Growth

Aged yellowedge grouper (*n*=1,556) represented fish harvested in offshore federal waters from all states in the GOM and by a variety of fishery independent and dependent gear types (Table 9). Yellowedge grouper sample collection over the last 26 years varied considerably by sector, gear and years sampled which made comparable comparisons over time difficult. Total length, weight and age data were summarized for each time period and gear type (Table 10). The majority of fish were collected from the commercial bottom longline (54%) and hand line (23%) fisheries. Considerably more fish were collected from the eastern GOM (*n*=1,083) than western GOM (*n*=594). In order to determine general biological parameters all age data were combined regardless

Table 9. Number of yellowedge grouper aged per sector (fishery dependent (*n*=1,204) and fishery independent (*n*=352)) and gear. BL – bottom longline, HL – hand line and other (bottom trawl (*n*=5), shrimp trawl (*n*=32), fish trap (*n*=2), unknown (*n*=2)). Otoliths that were classified as unreadable or preparation were excluded.

	Fishery Dependent			**Fishery Independent**			**Total**
Year	**BL**	**HL**	**Other**	**BL**	**HL**	**Other**	
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	235	2				249
1992	38	31					69
1999	55			41	1		97
2000	48	1		29		6	84
2001	325	49		28		9	411
2002				41		7	48
2003	188			55		7	250
2004	112	3		37		10	162
2005	63						63
Total	841	361	2	312	1	39	1,556
Percent	54.0	23.2	0.1	20.1	0.1	2.5	100.0

Table 10. Summary of life history statistics for yellowedge grouper from the Gulf of Mexico. Yellowedge grouper were collected in 1979-1989 (*n*=148), 1991-1992 (*n*=367) and 1999-2005 (*n*=1,172) using bottom longline (longline), hand line and other gear types (trawls, traps and unknown gear). Fishery independent and dependent samples combined. Results include the range (minimum-maximum), mean ±standard error (SE), median and sample sizes for each parameter: total length (mm), whole weight (kg) and age (years).

Time Period	Gear	Parameter	Range (min-max)	Mean±SE	Median	*n*
1979-1989	Longline	Total length	488-1050	731.4±13.8	690	97
		Weight	1.6-15.7	5.4±0.3	4.3	97
		Age	5-81	25.6±1.8	22	81
	Hand line	Total length	305-765	486.9±13.9	480	51
		Weight	0.4-2.7	1.3±0.1	1.3	28
		Age	4-11	5.6±0.3	5	42
1991-1992	Longline	Total length	460-1100	690.5±18.2	690	54
		Weight	1.3-16.0	4.1±0.4	3.7	41
		Age	3-50	14.8±1.2	13	50
	Hand line	Total length	290-1170	694.0±9.5	690	310
		Weight	0.2-17.8	4.6±0.2	3.7	308
		Age	2-77	18.4±0.9	14	266
	Other	Total length	640-890	743.3±75.4	700	3
		Weight	3.6-10.1	5.9±2.1	4.1	3
		Age	9-14	11.5±2.5	11.5	2
1999-2005	Longline	Total length	283-1148	682.1±4.4	669	1074
		Weight	0.4-16.0	4.3±0.1	3.8	588
		Age	2-85	16.4±0.3	14	1022
	Hand line	Total length	262-924	536.7±15.9	527	59
		Weight	0.5-6.0	2.4±0.7	1.4	9
		Age	2-26	10.2±0.7	9	54
	Other	Total length	107-1076	273.8±38.1	176	39
		Weight	0.02-11.1	1.0±0.4	0.8	38
		Age	0-38	4.7±1.5	1	39

of sector (fishery independent or dependent), gear, location or year to illustrate yellowedge grouper size-at-age. Ages were determined for 92.2% (*n*=1,556) of yellowedge grouper. Aged fish ranged in length from 107 to 1,160 mm TL (mean=667.6, SE=4.25), weighed 0.2 to 17.8 kg (mean=4.1, SE=0.08) and averaged 16.3 years old (SE=0.29, range=0-85 years). Although yellowedge grouper can live in excess of 85 years only a small percent of fish were found in the older age classes and the numbers of older fish have decreased over time. During 1979-1989 and 1991-1992, 95% of fish were 51 and 48 years old or less, respectively. While in 1999-2005 the number of older fish dropped considerably and 95% of yellowedge grouper were 33 years old or less.

The majority of otoliths were classified as readable (*n*=1,133) according to the readability scale. Only 45 otoliths were considered unreadable and 86 were excluded due to breakage or preparation (Figure 4). Otolith classification varied on an individual fish basis, for example the otolith of a 12 year old fish could be classified as good, readable, or difficult (Figure 5). The cumulative percent of readings in exact agreement between both readers was 13%, agreement ±1 was 37.1%, and agreement ±2 was 53.4% while percent agreement increased to 78.5% ±5 years. Differences of greater than six years occurred for 21.5% of otoliths aged. The overall APE for all yellowedge grouper aged was 11.9% with a CV of 16.8%. Reader precision increased throughout the duration of the study as readers gained experience. The APE for the first 40% of otoliths aged was 14.7% (CV=20.8%) verses 10.0% (CV=14.1%) for the remaining otoliths.

Otolith Weight Comparisons. No significant difference was found between the weight of the left and right otoliths (t_{275}=-0.56, *P*=0.574) indicating either otolith could

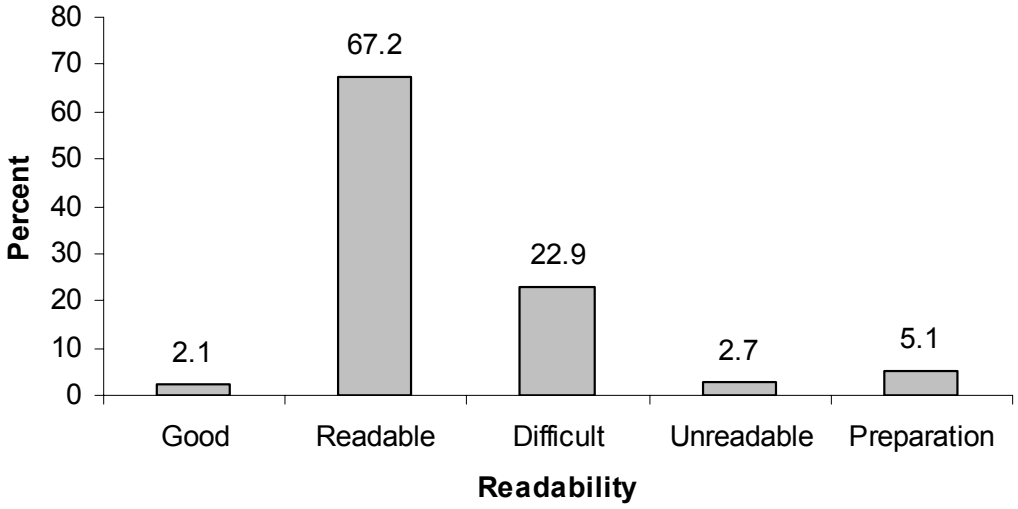


Figure 4. Number of aged yellowedge grouper based on readability classification scale (Kau and Tanaka, 1984).

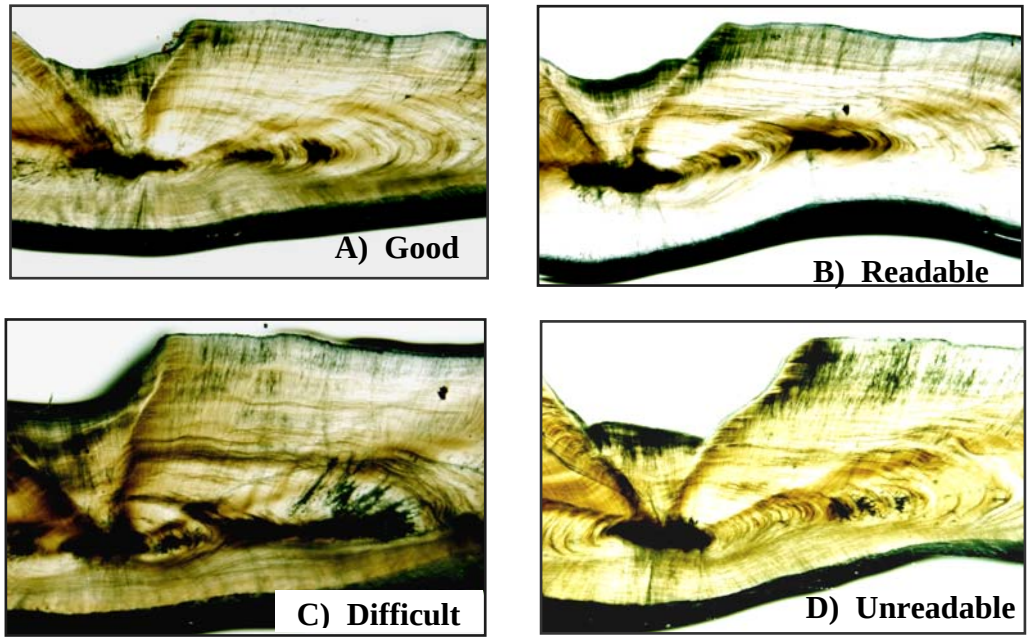


Figure 5. Yellowedge grouper sectioned otoliths of 12 year old fish illustrating the differences between readability classification codes A) Good, B) Readable, C) Difficult, D) Unreadable. The unreadable section is of a similar sized fish as the fish otolith classified as good. Photographs are of the ventral side of the otolith taken at 20x magnification. Readability classification scale based on Kau and Tanaka (1984).

be used for age analysis. A positive linear relationship was observed between fish age and otolith weight ($F_{1,1283}$=6925.63, P<0.001, R^2=0.844; Figure 6). Conversely otolith weight could be used as a reliable predictor of age using the following equation: Age=14.866*otolith weight+0.944. When compared to observed ages determined by otolith growth increment counts the above equation averaged the same age estimate as the observed age and most ages were over estimated by only one year.

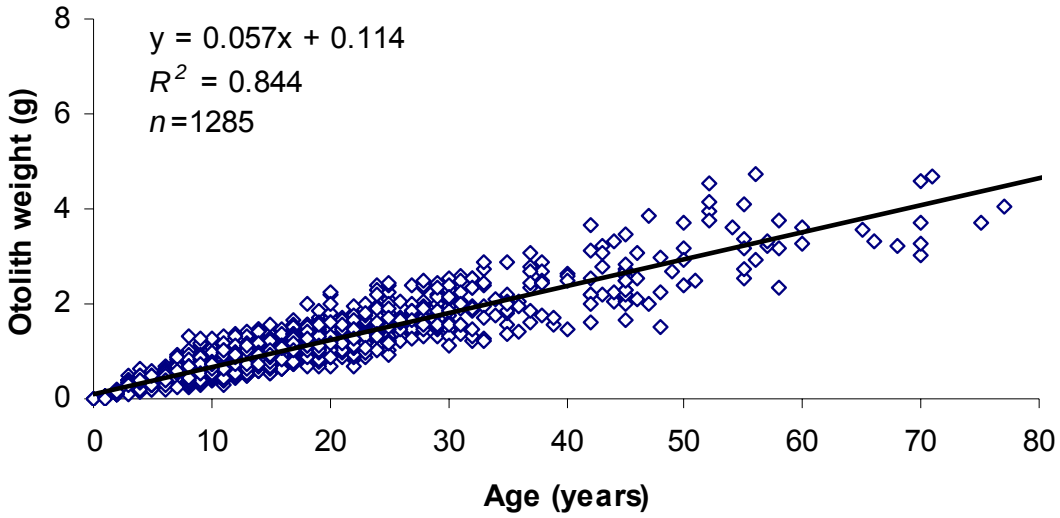


Figure 6. Relationship between yellowedge grouper age and otolith weight.

Age-Length Structure. Age-length frequencies were produced by sector and gear (Figure 7A, B), year of capture (time period): 1) 1979-1989, 2) 1991-1992 and 3) 1999-2005) (Figure 8) and Zone (Figure 9). Age data was not normally distributed (K-S; P<0.001) and transformations did not normalize the data in most cases, therefore, nonparametric tests were often applied for statistical analysis and median rather than mean ages reported. No significant difference was found between the ages

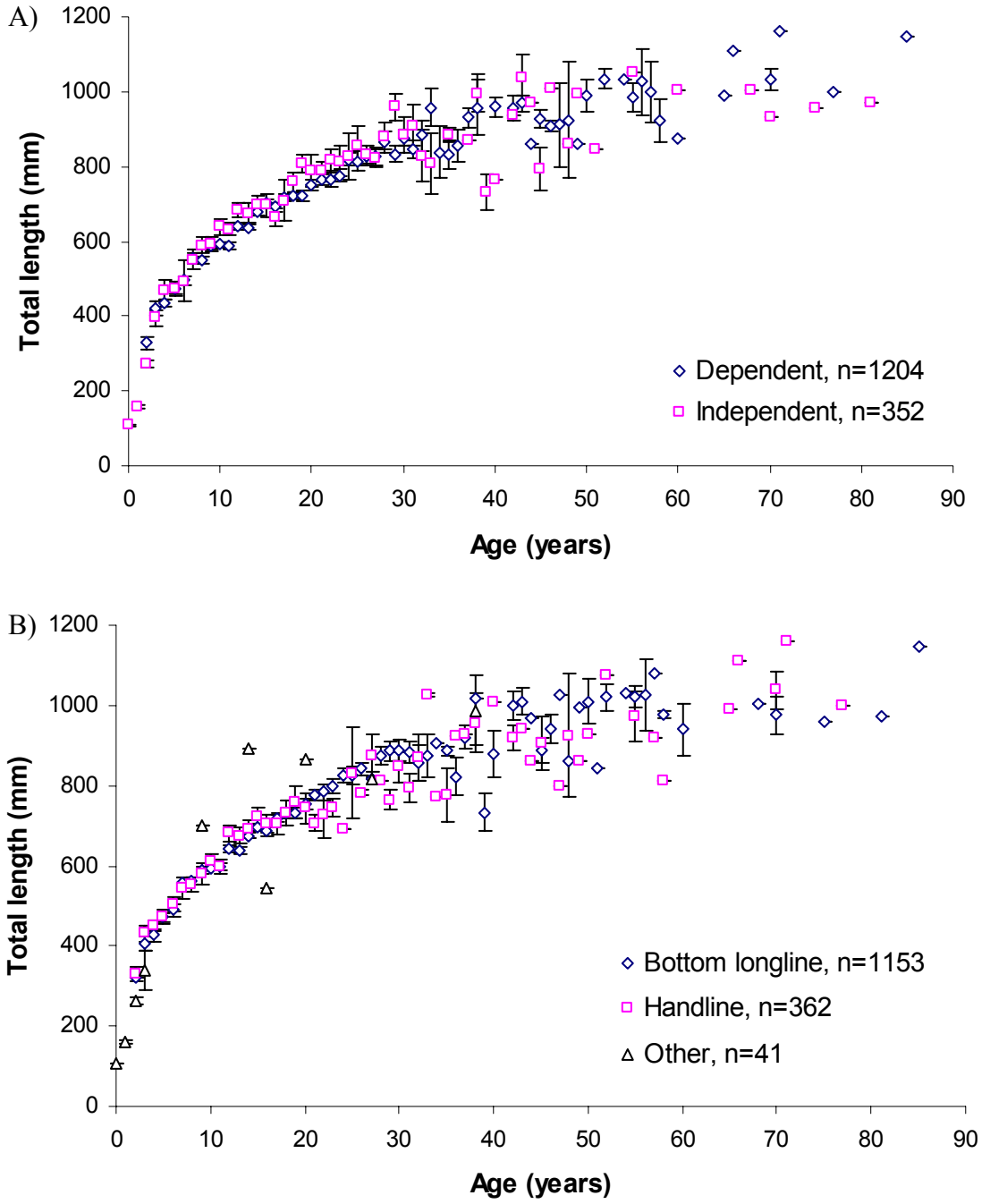


Figure 7. Mean size-at-age of yellowedge grouper collected from 1979-2005 by A) sector (fishery dependent and independent) and B) gear. Gear used included bottom longline (vertical drift lines and off-bottom longlines), hand line and other (trawls and fish traps). Error bars represent ±1 standard error.

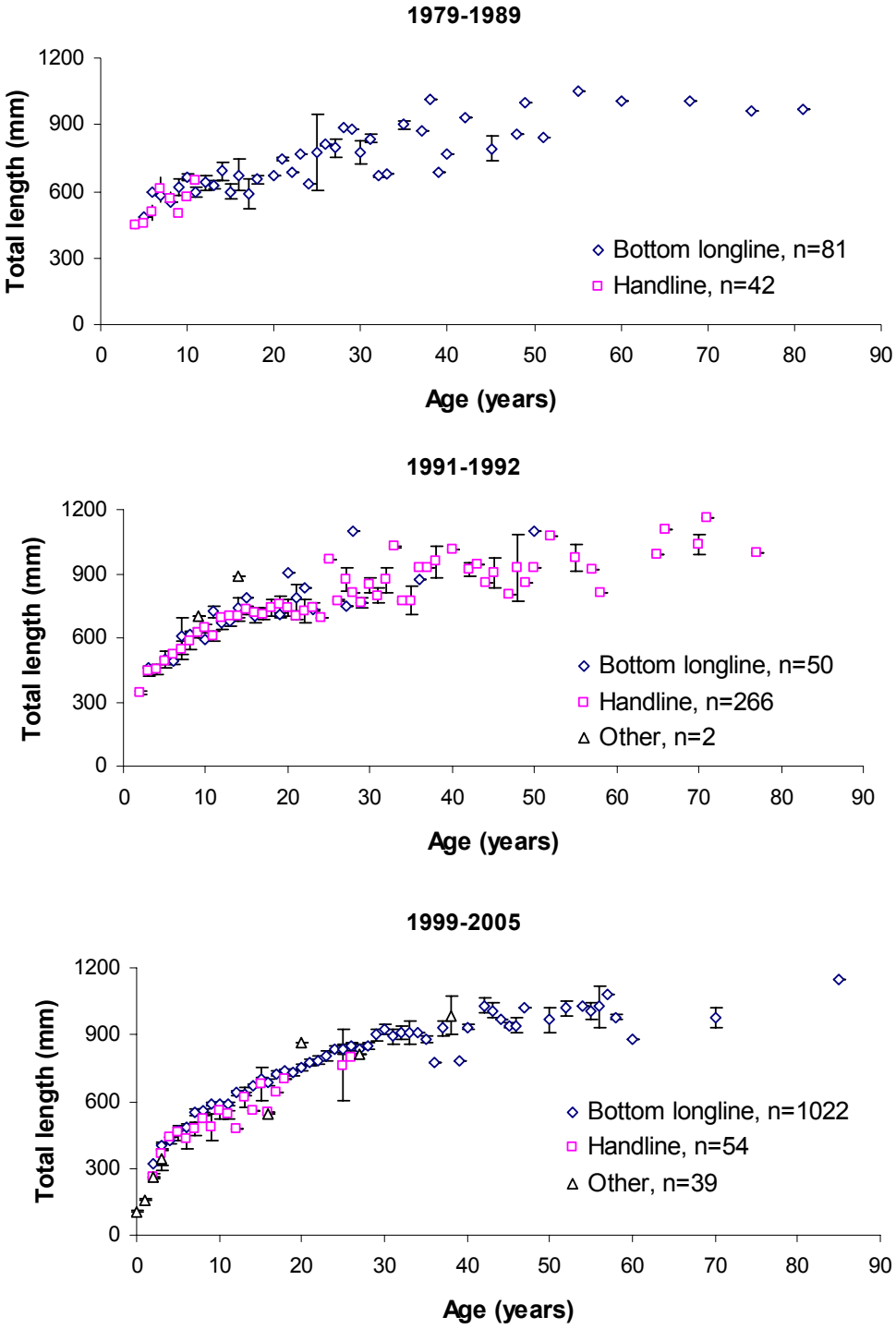


Figure 8. Yellowedge grouper mean size-at-age per gear type over time. Gear used included bottom longlines (off-bottom longlines and vertical drift lines), hand lines, and other (trawls and fish traps). Fishery dependent and independent data combined. Error bars represent ±1 standard error.

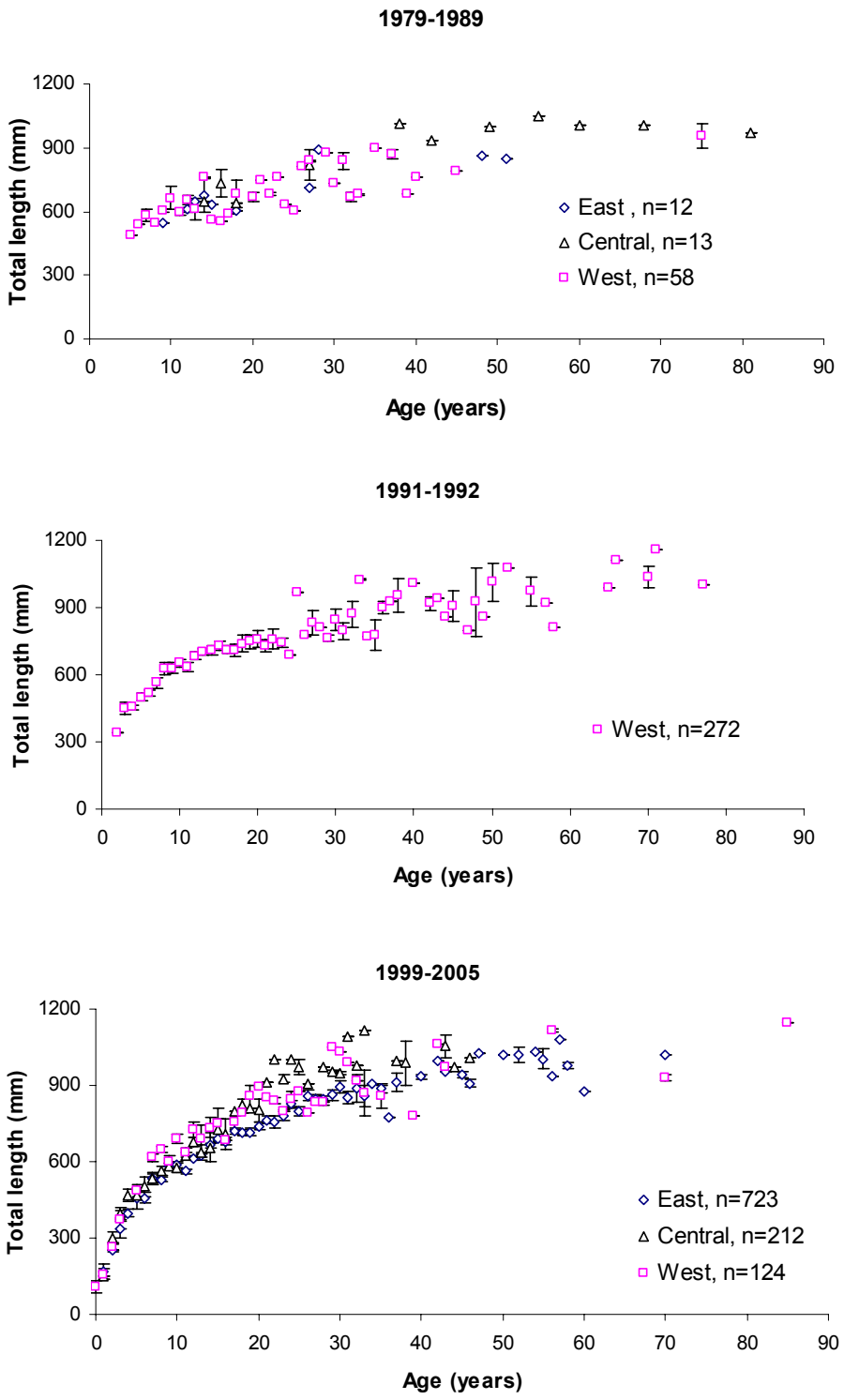


Figure 9. Mean size-at-age over time of yellowedge grouper captured in the east, central and west Gulf of Mexico. All gear types and fishery dependent and independent data combined. Error bars represent ±1 standard error.

(Mann-Whitney, Z=-1.383, P=0.167) or total lengths (Mann-Whitney, Z=-0.974, P=0.330) of yellowedge grouper collected by fishery dependent verses independent sectors, therefore, samples were combined for remaining analyses. A significant difference in median age (Kruskal-Wallis, H=99.36, P<0.001) and length (Kruskal-Wallis, H=83.75, P<0.001) was detected between each different gear type used (Dunn's comparison, P<0.05). Longlines collected the oldest (median=14 years) and largest (median=673 mm) fish followed by hand lines (median=12 years, 640 mm) and other gear (median=1 year, 177 mm) which consisted primarily of trawling gear.

A significant difference was found between the length-frequency distributions of yellowedge grouper sampled during 1979-1989 and 1991-1992 (K-S, Z=1.85, P=0.002) and between 1991-1992 and 1999-2005 (K-S, Z=1.98, P<0.001; Figure 10).

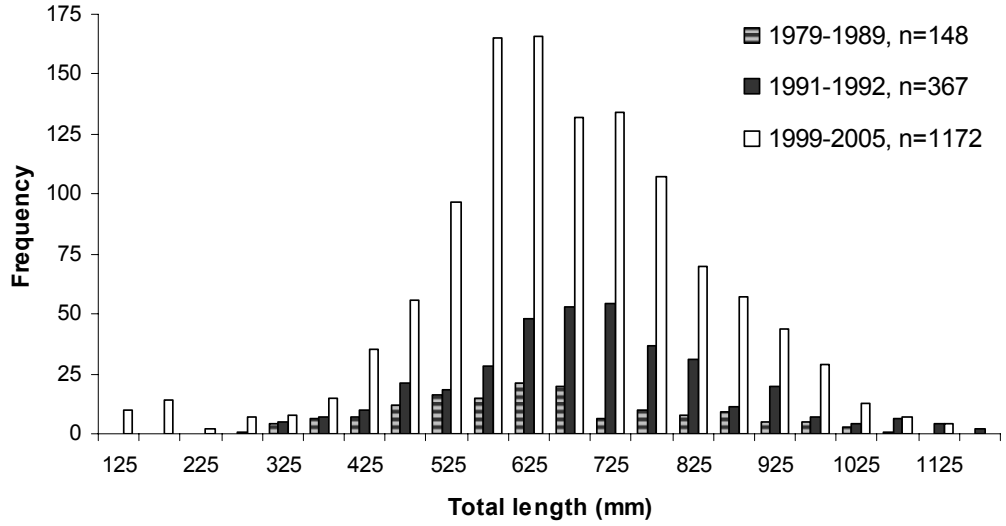


Figure 10. Length-frequency of yellowedge grouper collected per time period. Total length represents the midpoint of each size class. Yellowedge grouper were collected using bottom longlines, hand lines, trawls and traps.

No difference was found between time periods one and three (K-S, Z=1.261, P=0.083). Yellowedge grouper collected in 1991-1992 were larger (median=690 mm) than those from 1979-1989 (median=634 mm) and 1999-2005 (median=655 mm) (Kruskal-Wallis, H=13.82, P<0.001). However, there was no significant difference (Kruskal-Wallis, H=0.056, P=0.756) between the median ages (Figure 11) of yellowedge grouper from 1979-1989 (median=12 years), 1991-1992 (median=14 years) and 1999-2005 (median=14 years).

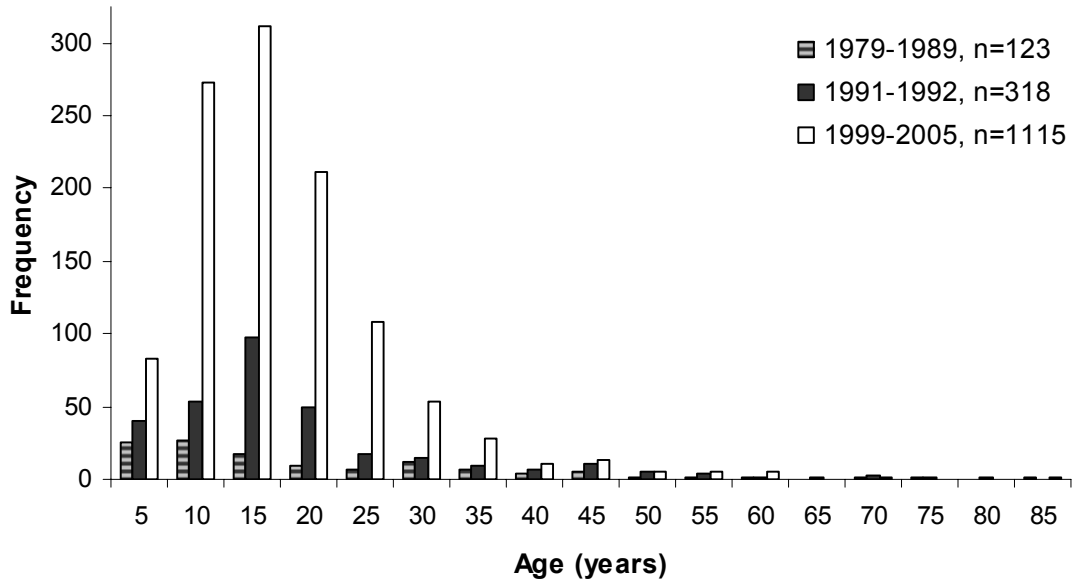


Figure 11. Age-frequency of yellowedge grouper collected per time period. Age represents the maximum age in each size class. Yellowedge grouper were collected using bottom longlines, hand lines, trawls and traps.

Bottom longline gear was the primary gear used for yellowedge grouper collection. Since significant age and length differences of fish existed between gear types, TL and age of fish collected using only bottom longline gear were also evaluated

over time. Bottom longline data were normally distributed (K-S test, $P>0.05$); therefore, parametric statistics were applied. Significant differences between both TL (ANOVA, $F_{2,1222}=5.25$, $P=0.005$) and age (ANOVA, $F_{2,1150}=22.20$, $P<0.001$) were observed between time periods. Yellowedge grouper collected using bottom longlines were significantly larger in 1979-1989 (Bonferroni, $P=0.004$; $n=97$, mean=731.4) than in 1999-2005 ($n=1,074$, mean=682.1). However, no significant difference was found between 1979-1989 and 1991-1992 (Bonferroni, $P=0.284$; $n=54$, mean=690.5) or between 1991-1992 and 1999-2005 (Bonferroni, $P=1.000$). Significant differences in age occurred during 1979-1989 ($n=81$, mean=26 years) and both 1991-1992 ($n=50$, mean=15 years) and 1999-2005 ($n=1,074$, mean=16 years; Games-Howell, $P<0.001$). However, no difference in age was found between 1991-1992 and 1999-2005 (Games-Howell, $P=0.441$).

Age data from the eastern and western GOM were not normally distributed and TL data violated the assumption of homogeneity of variance (Levene's test, $F=135.62$, $P<0.001$), therefore, nonparametric tests were used to compare the two regions. No significant difference was found between the median TL (Mann-Whitney, $Z=-1.125$, $P=0.261$) or median age (Mann-Whitney, $Z=-1.080$, $P=0.280$) of yellowedge grouper collected in the eastern and western GOM. Although differences were not found, yellowedge grouper were larger in the western GOM (median=726 mm) than in the eastern GOM (median=650 mm), however, median ages were similar, 13.5 years and 14.0 years for the western and eastern GOM, respectively. Considerably fewer aged samples were available from the western GOM ($n=132$) than the eastern GOM ($n=981$) which may have influenced statistical analysis. Although not enough samples were

available for statistical comparison, yellowedge grouper mean size at age appeared larger in the western GOM than in the eastern GOM (Figure 12) for nearly every age class.

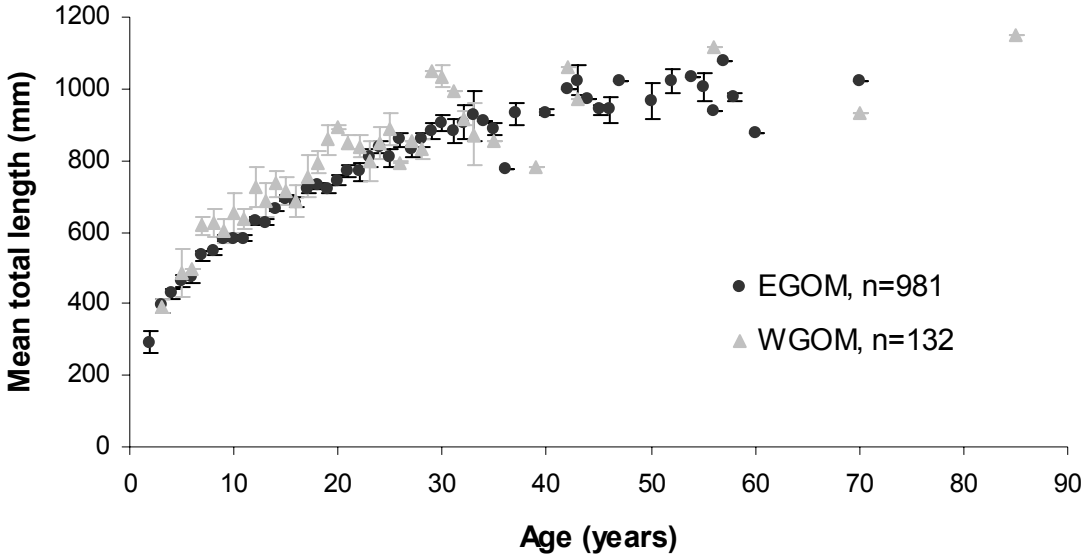


Figure 12. Mean total length at age of yellowedge grouper collected in the eastern (E) and western (W) Gulf of Mexico (GOM) during 1999-2005. Error bars represent ±1 standard error.

von Bertalanffy growth curves. Von Bertalanffy growth curves were fitted to observed mean length at age data for all yellowedge grouper combined (Figure 13), each time period (Figure 14) and eastern and western GOM fish from 1999-2005 (Figure 15; Table 11). Overall growth (K) was slow (K=0.063) resulting in a theoretical asymptotic length (L_∞) of 970 mm TL. Yellowedge grouper growth rate appeared to increase slightly from 1979-1989 (K=0.042) to 1991-1992 (K=0.058) but remained comparable through 1999-2005 (K=0.063). Yellowedge grouper recently collected in the western GOM

appeared to grow two times faster (K=0.100) than fish from the eastern GOM (K=0.048). Significantly different von Bertalanffy growth curves were observed between each time period (LRT, χ^2=100.59, P<0.001) and region (LRT, χ^2=94.639, P<0.001).

Multiple pair-wise comparisons were conducted to determine which time periods and parameters differed and which parameters differed between the two regions (Table 12). Significant differences were found between all three time periods (P<0.001). Multiple comparisons between time periods 1979-1989 and 1991-1992 indicated no significant differences between parameters (K, L_∞ and t_0) suggesting that specific parameter differences were too slight to be detected on an individual basis. Comparisons between 1979-1989 verses 1999-2005 and 1991-1992 verses 1999-2005 indicated significant differences between t_0. Differences between K and L_∞ did not occur indicating growth rate did not change over time. Multiple comparisons between the eastern and western GOM indicated significant differences between all three parameters (K, L_∞ and t_0) suggesting that more than one population of yellowedge grouper may inhabit the GOM.

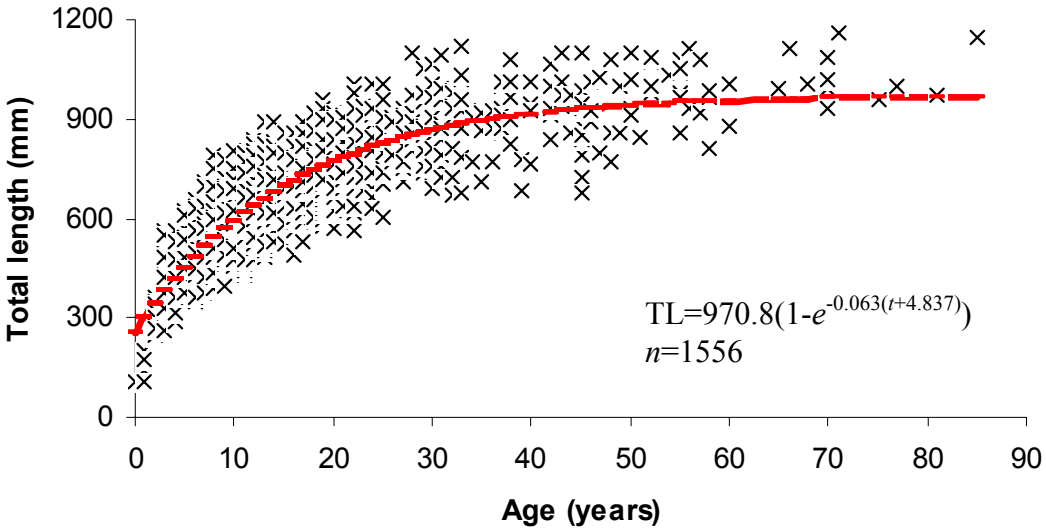


Figure 13. Age and growth of yellowedge grouper collected from 1979-2005. The solid line represents all the data fitted to the von Bertalanffy growth curve. Both sectors and all gear types were combined.

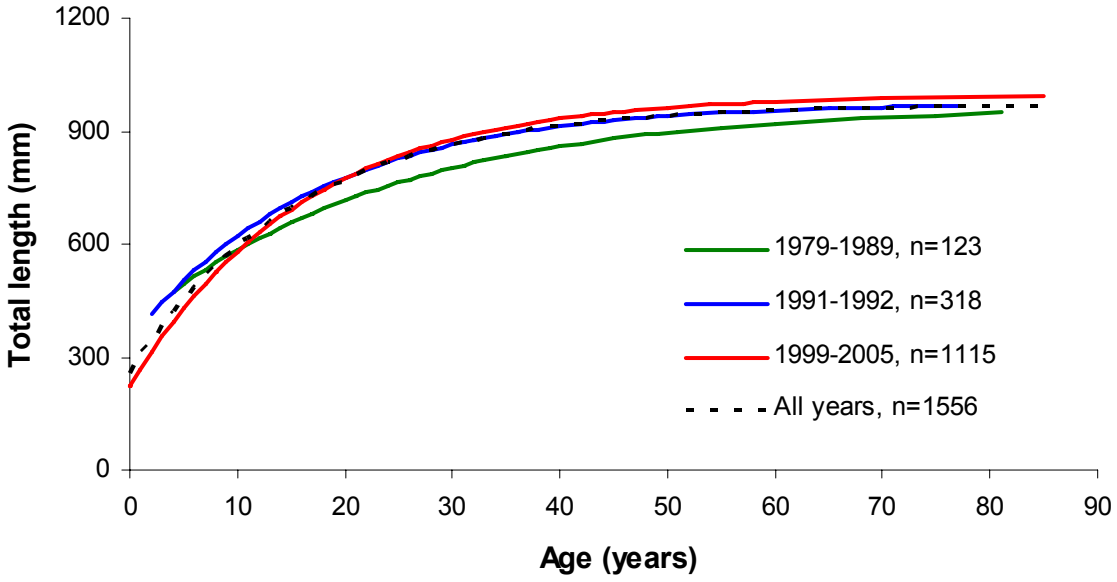


Figure 14. Results of von Bertalanffy growth curves for yellowedge grouper collected 1979-2005.

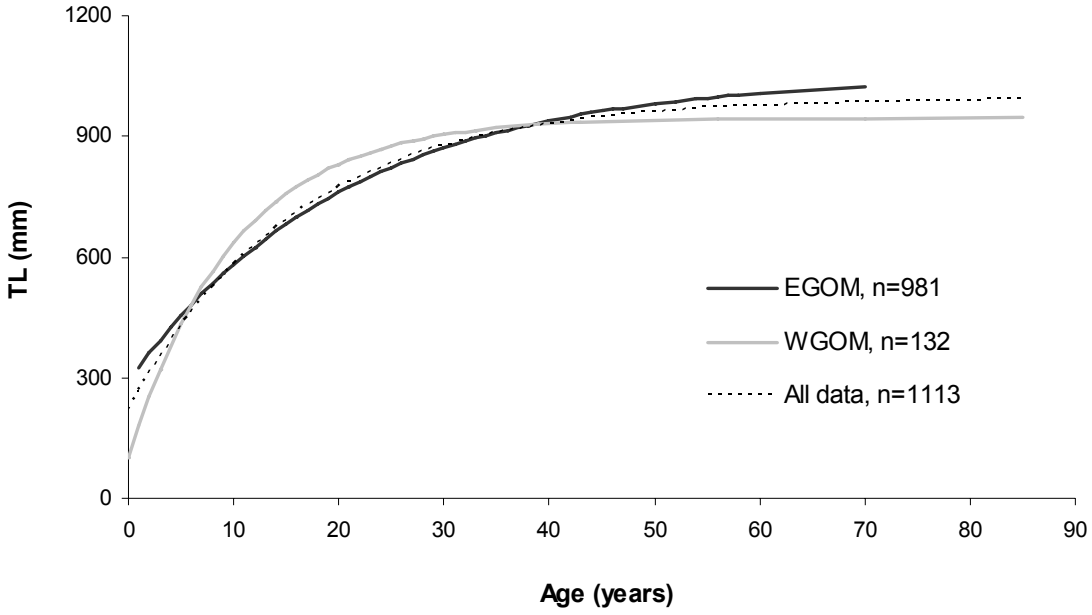


Figure 15. Results of von Bertalanffy growth curves for yellowedge grouper collected in the eastern (E) and western (W) Gulf of Mexico during1999-2005.

Table 11. Results of von Bertalanffy growth curves. Comparisons by year included fish collected throughout the entire Gulf of Mexico (GOM), EGOM and WGOM refer to yellowedge grouper collected in the eastern (E) and western (W) GOM during 1999-2005. Source refers to the data used in the analysis, n is the number of samples, L_∞ 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)	n	L_∞	K	t_0	R^2
All years combined	107-1160	0-85	1556	970.8	0.063	-4.84	0.76
1979-1989	335-1050	4-81	123	966.9	0.042	-11.87	0.67
1991-1992	290-1160	2-77	318	974.6	0.058	-7.542	0.73
1999-2005	107-1148	0-85	1115	995.5	0.063	-3.996	0.78
EGOM	141-1119	1-70	981	1048.4	0.048	-6.700	0.75
WGOM	107-1148	0-85	132	945.8	0.100	-1.144	0.90

Table 12. Results of von Bertalanffy growth curve comparisons. Comparisons by year included fish collected throughout the entire Gulf of Mexico (GOM), EGOM and WGOM refer to yellowedge grouper collected in the eastern (E) and western (W) GOM during 1999-2005. Coincident refers to the combined data curve, L_∞ is the theoretical maximum length, K is the growth rate coefficient and t_0 is the theoretical age at length zero. * Indicates a significant difference.

Time period	Parameter	χ^2	df	P
1979-1989 vs. 1991-1992	Coincident	22.16	3	<0.001*
	L_∞	0.02	1	0.896
	K	1.61	1	0.205
	t_0	2.15	1	0.143
1979-1989 vs. 1999-2005	Coincident	52.68	3	<0.001*
	L_∞	0.27	1	0.606
	K	3.50	1	0.061
	t_0	13.60	1	<0.001*
1991-1992 vs. 1999-2005	Coincident	62.11	3	<0.001*
	L_∞	0.56	1	0.453
	K	0.52	1	0.473
	t_0	12.14	1	<0.001*
EGOM vs. WGOM	Coincident	94.64	3	<0.001*
	L_∞	10.22	1	<0.001*
	K	43.50	1	<0.001*
	t_0	71.32	1	<0.001*

Reproduction

Gonad sample acquisition. A total of 605 gonads were collected from 1999-2005 by fishery dependent (*n*=365) and fishery independent (*n*=240) sources. The majority (96.4%) of the samples were collected using commercial (*n*=357) and scientific (*n*=226) bottom longline gear. The remaining twenty-two samples were collected with commercial hand lines (*n*=8) and scientific shrimp (*n*=10) and high opening bottom trawls (*n*=4). Samples were collected in all months except January (Table 13), however, the bulk of samples were collected between April and September.

Table 13. Number of gonads collected per month from 1999-2005 by fishery dependent and fishery independent sources.

Source	Month											Total
	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	
Dependent	60	23	111	105	39	12	14	1	0	0	0	365
Independent	0	3	0	44	26	14	99	35	7	7	5	240
Total	60	26	111	149	65	26	113	36	7	7	5	605

Size frequency distribution. Pooled data from all sources were normally distributed (K-S test; *n*=605, *P*=0.302); yellowedge grouper ranged in size from 141-1,100 mm (mean=684.4 mm). Mean sizes of fishery dependent (mean=691.4 mm) samples were not significantly different from independent (mean=673.7 mm) samples (ANOVA, $F_{1,603}$=1.96, *P*=0.162; Figure 16). Therefore, all reproductive samples were pooled together to represent all size classes for the determination of reproductive life history parameters.

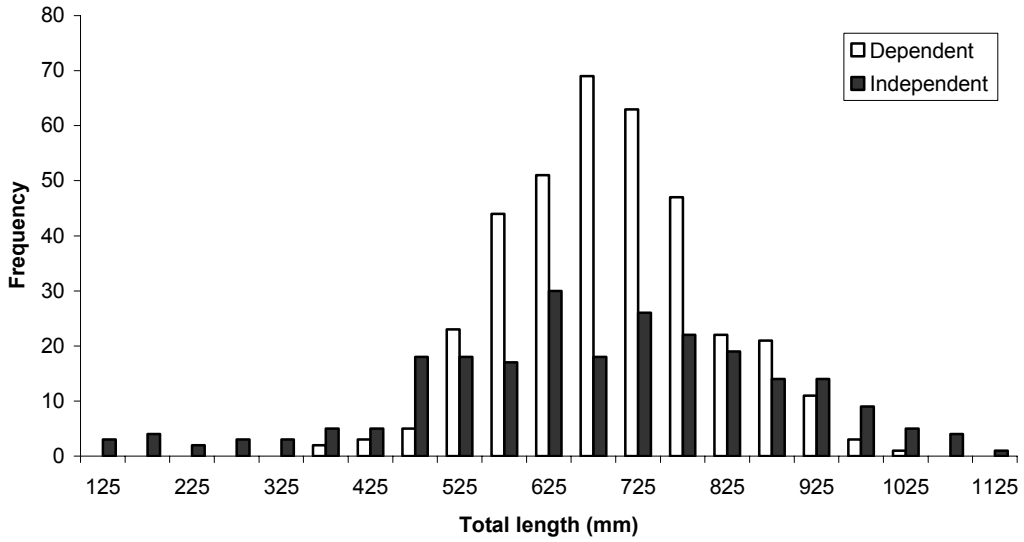


Figure 16. Size frequency distributions of fishery dependent (n=365) and fishery independent (n=240) yellowedge grouper used for reproductive data analysis. Total length represents the midpoints of each size class.

Fresh and fixed gonad weight comparison. A paired samples t-test determined a significant difference (n=18, t=3.19, P=0.005) between the weights of fresh and fixed gonads for both males and females. Fixed gonads weighed an average of 11-22% less than fresh gonads; weight change was heavily influenced by sexual stage. Therefore, fixed gonad weights for both sexes of immature, early maturation, mid maturation, regenerating, unknown maturation stages and transitional fish were increased by the mean percent change of 11.15%. Gonad weight of fish classified as late maturation or regressing and fish beginning final oocyte maturation were adjusted using linear regression (Fresh weight = 1.834 + 1.103 (Fixed weight), n=13, R^2=0.98, P<0.001). Yellowedge grouper gonads containing hydrated oocytes experienced the greatest differences between fresh and fixed weights and were adjusted using linear regression as follows; Fresh weight = 3.945 + 1.242 (Fixed weight), n=2, R^2=1.00.

Homogeneity of oocyte development. No significant difference (P>0.05) was found between lobes and sections for each oocyte type indicating homogeneous distribution throughout the gonad (Table 14). Therefore, only one sample, primarily from the posterior, was taken from each gonad for histological analysis.

Table 14. Results of linear mixed models (LMM) using the compound symmetry covariance structure to illustrate homogeneity of oocyte types throughout the gonad. Each gonad was divided into two lobes (Lobe; left, right) and three sections (Section; anterior, center, posterior). *Indicates no significant difference.

Oocyte type	Source	Numerator df	Denominator df	*F*	*P*
Cortical alveoli	Intercept	1	9.0	48.12	.000
	Lobe	1	47.0	0.26	.614*
	Section	2	47.0	0.05	.947*
Vitellogenic	Intercept	1	9.0	169.58	.000
	Lobe	1	47.0	0.07	.795*
	Section	2	47.0	1.66	.201*
Final oocyte	Intercept	1	9.0	5.84	.039
maturation	Lobe	1	47.0	2.27	.138*
(FOM)	Section	2	47.0	0.12	.888*

Reproductive summary. Females (n=468) were significantly smaller than males (n=134; ANOVA, $F_{2,602}$=135.85, P<0.0001, Bonferroni, P<0.0001). Females ranged from 141-980 mm (mean=638.4, SE=6.16) while males ranged from 575-1,100 mm (mean=843.2, SE=8.96). The mean TL (772.7 mm, SE=39.57, range=703-840 mm) of transitional fish (n=3) was not significant from that of females (Bonferroni, P=0.206) or males (Bonferroni, P=1.000) (Figure 17). Females weighed 0.04-14.0 kg (mean=3.36, SE=0.090) while males weighed 2.1-16.0 kg (mean=7.2, SE=0.253) and transitional fish weighed 3.8-6.1 kg (mean=5.3 kg, SE=0.77).

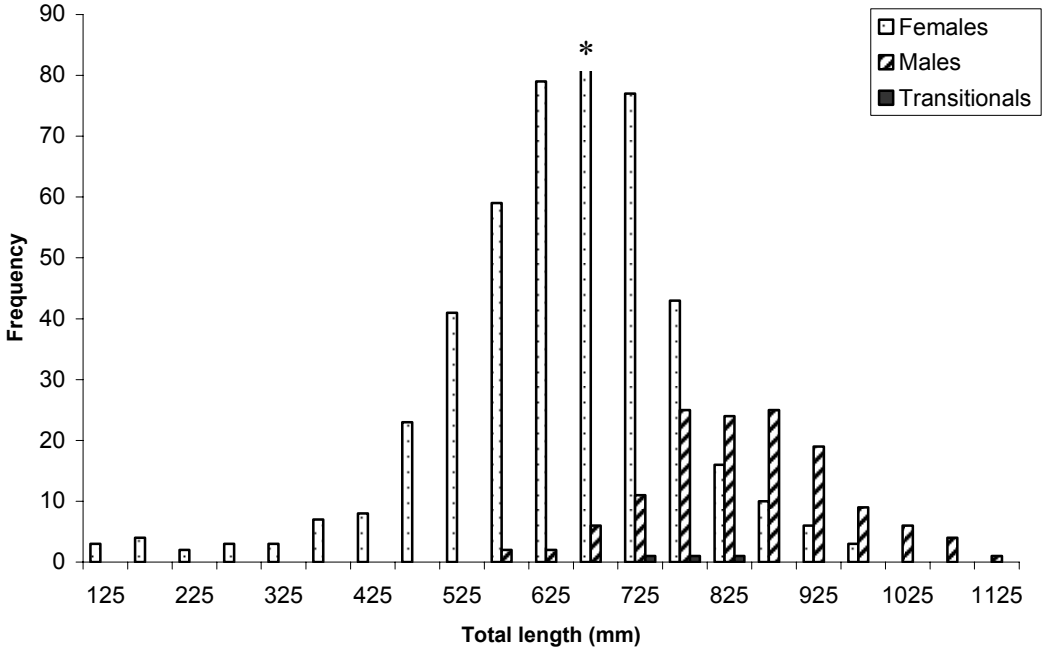


Figure 17. Length-frequency of female (n=468), male (n=134) and transitional (n=3) yellowedge grouper collected during 1999-2005. *Size class at which all females have reached length of sexually maturity. Data labels indicate the midpoint of the size classes.

Reproductive and age (range 1-70 years, mean=15.3 years, SE=0.33) information was available for 591 yellowedge grouper. Mean ages of females (12.8 years, SE=0.27, range 1-33 years) were considerably lower than males (23.7 years, range 8-70 years, SE=0.79) and transitional fish (20.7 years, SE=5.18, range 15-31 years). Age data was not normally distributed, therefore a Kruskal-Wallis test was used to determine that median ages differ significantly by sex (H_2=173.69, P<0.001). The median age of females (12 years) was significantly less than the median age of males (22 years) (Dunn's Method, Q=13.11, P<0.01). There was no significant difference between the median age of transitional fish (16 years) and females or males (Dunn's Method, P>0.05).

Size and age at maturity. Histological examination of yellowedge grouper ovaries

revealed all sexual maturation stages, from immature through regenerating, (Figure 18 A-G) were present. Immature females ranged in age from 0-16 years old and 141-639 mm TL. Females began reaching sexual maturity at age six and all females were sexually mature by age seventeen. Size at sexual maturity began at 475 mm TL and all females were mature by 640 mm TL (Figure 19). Many immature and mature females contained precocious spermatogenic cysts which appeared to remain dormant until sexual transition.

Length and age at 50% percent maturity of all females were 512 mm TL (95% CI=410-692) and 7.0 years (95% CI=5-9), respectively. Length and age at 50% percent maturity of only active females were 543 mm TL (95% CI=429-754) and 8.2 years (95% CI=6-11), respectively (Figure 20 A, B). Differences were observed when size and age (Figure 21 A, B) at maturity were analyzed according to region. Yellowedge grouper from the western GOM (*n*=69) reached maturity at larger sizes and similar ages than those in the eastern GOM (*n*=394). Length at 50% percent maturity of all western GOM females was 533 mm TL (95% CI=303-1,228) and 594 mm TL (95% CI=240-1,234) for only active females (*n*=48). Length at 50% percent maturity of all eastern GOM females was 511 mm TL (95% CI=401-722) and 544 mm TL (95% CI=416-776) for active females (*n*=245). Age at 50% maturity was 6.6 years (95% CI=6-10) and 7.1 years (95% CI=4-16) for all western and eastern GOM females, respectively. Ages at which 50% of active females reached maturity were 8.8 years (95% CI=5-73) in the western GOM and 8.1 years (95% CI=6-11) in the eastern GOM. The overlapping confidence intervals for both size and age indicated that although differences were observed they were not conclusive.

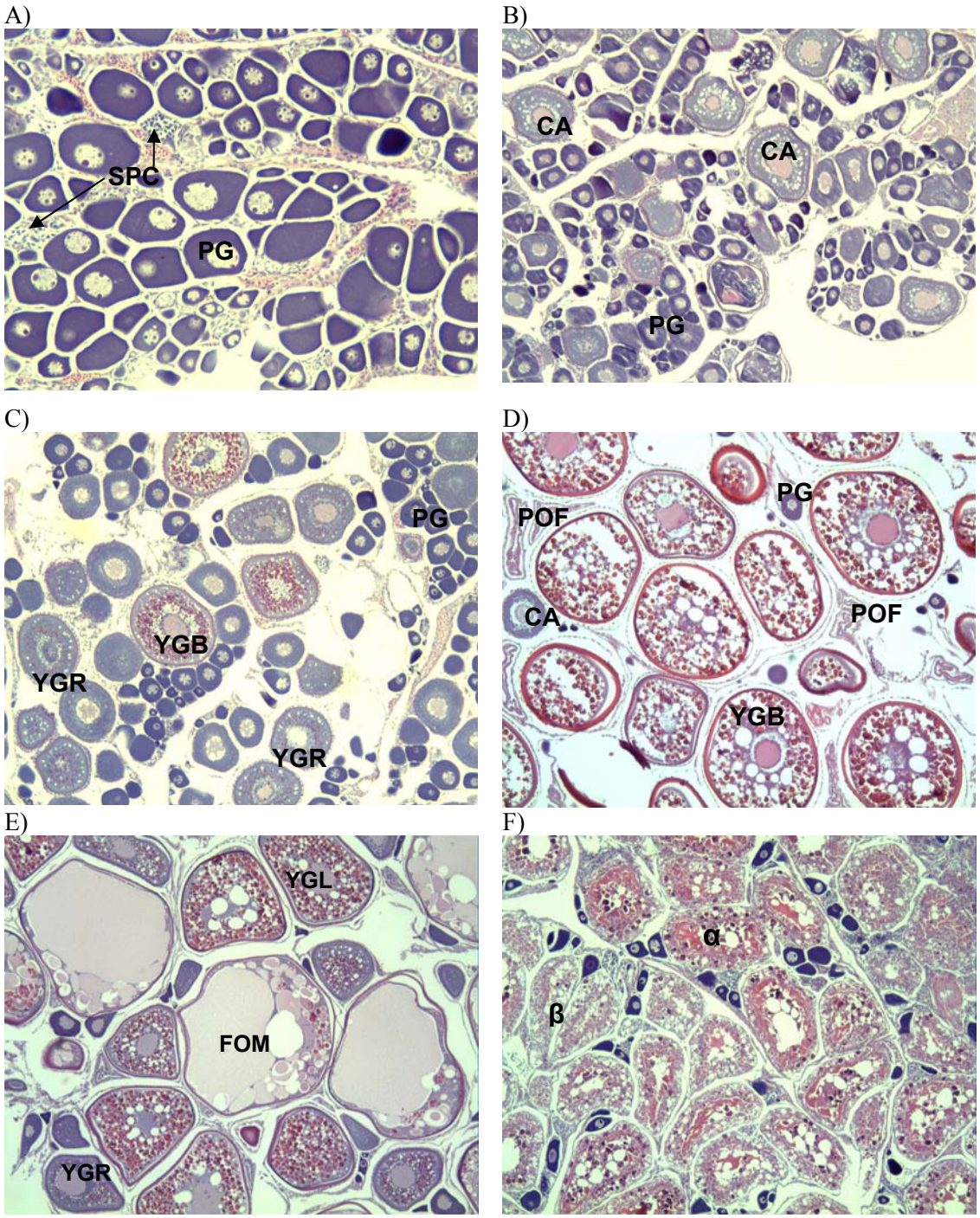


Figure 18. Histological sections of ovarian tissue from yellowedge grouper in various maturation classes. A) Immature–primary growth (PG) oocytes only and occasionally spermatogenic cysts (SPC). B) Early maturation–cortical alveoli oocytes (CA) present. C) Mid-maturation–yolk granule (YGR) and yolk globular oocytes (YGB) present. D) Late maturation–yolk globular oocytes predominate, 12 hour postovulatory follicles present (POF). E) Final oocyte maturation (FOM)–yolk coalescence occurs. F) Regressing–atresia predominates; alpha (α) and beta (β). Photograph A) 200x magnification, remaining photographs 80x magnification.

G)

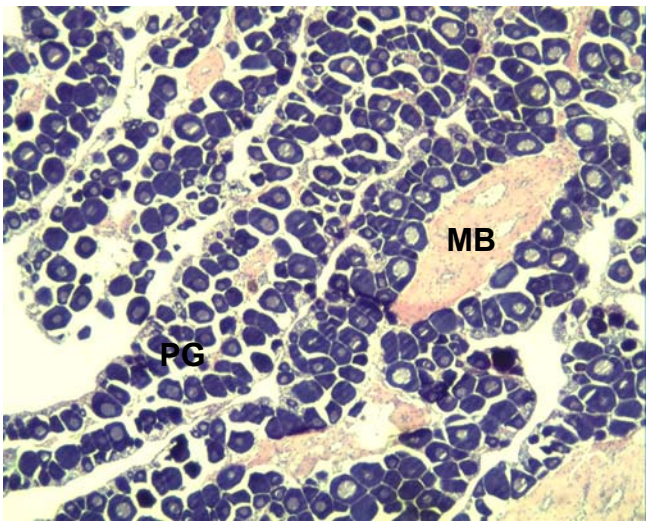


Figure 18 (continued). Histological sections of ovarian tissue from yellowedge grouper in various maturation classes. G) Regenerating–only primary growth (PG) oocytes occur, muscle bundles (MB) indicate prior spawning (80x magnification).

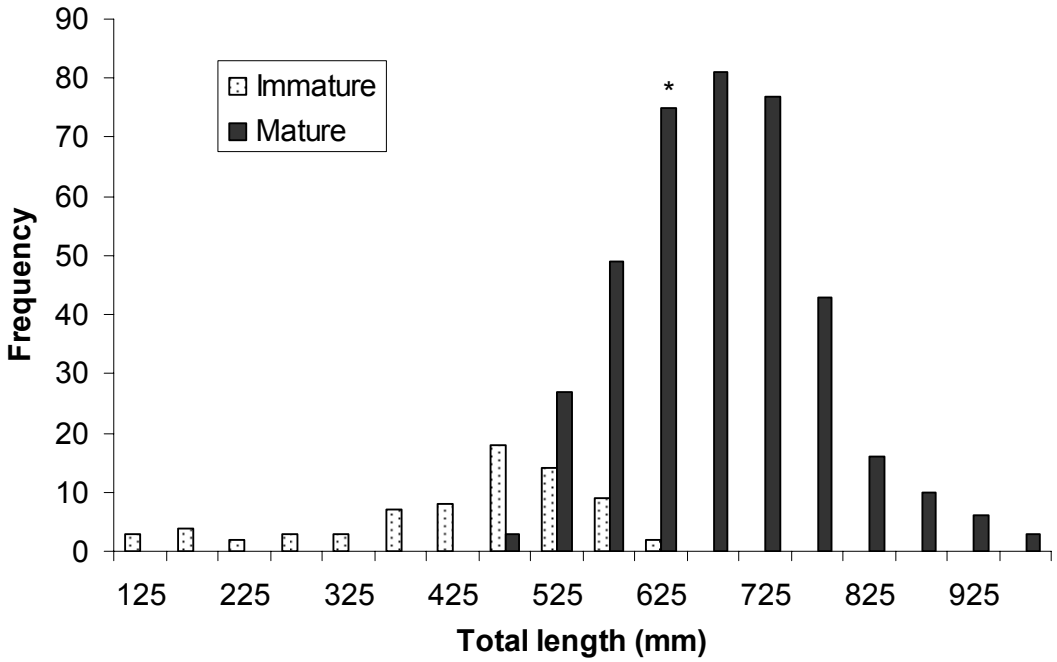
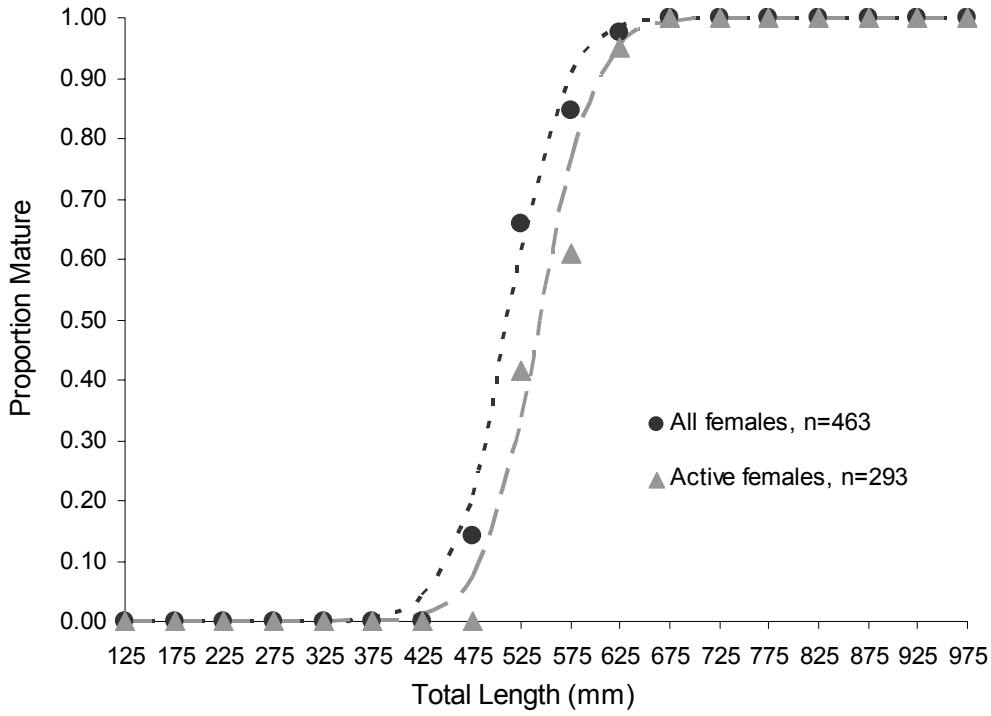


Figure 19. Length-frequency of immature (*n*=73) and mature (*n*=390) female yellowedge grouper collected during 1999-2005. *All females have reached length of sexually maturity by 640 mm total length. Data labels indicate the midpoint of the size classes.

A)



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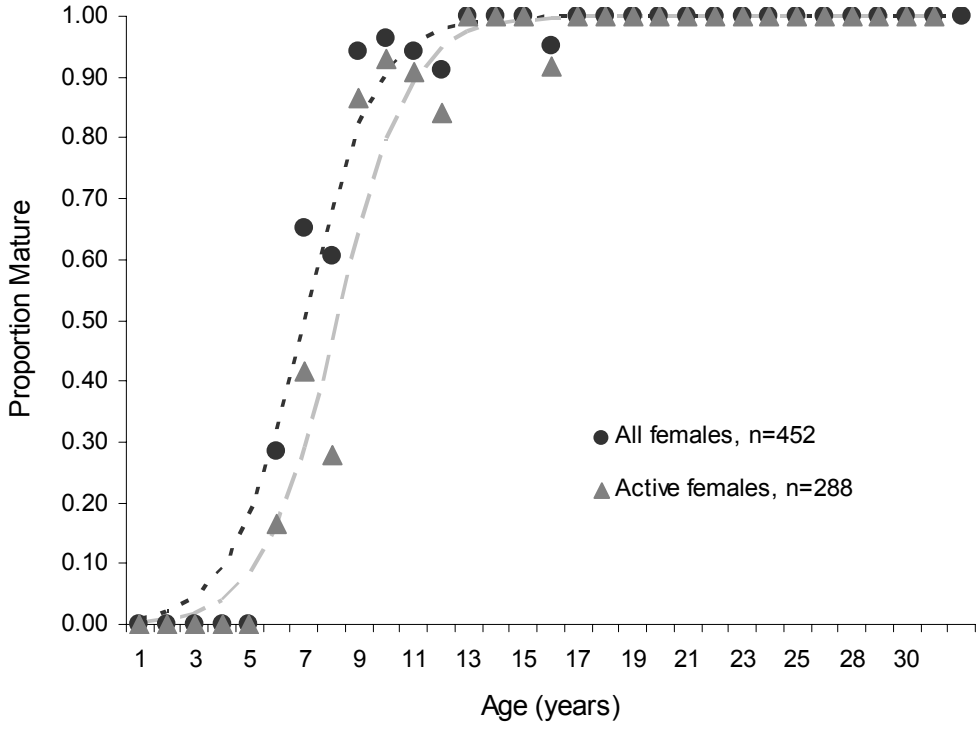

Figure 20. Maturity estimates calculated for A) total length and B) age at 50% mature for female yellowedge grouper. Active females were classified as those with vitellogenic oocytes present. Total length represents the midpoint of each size class.

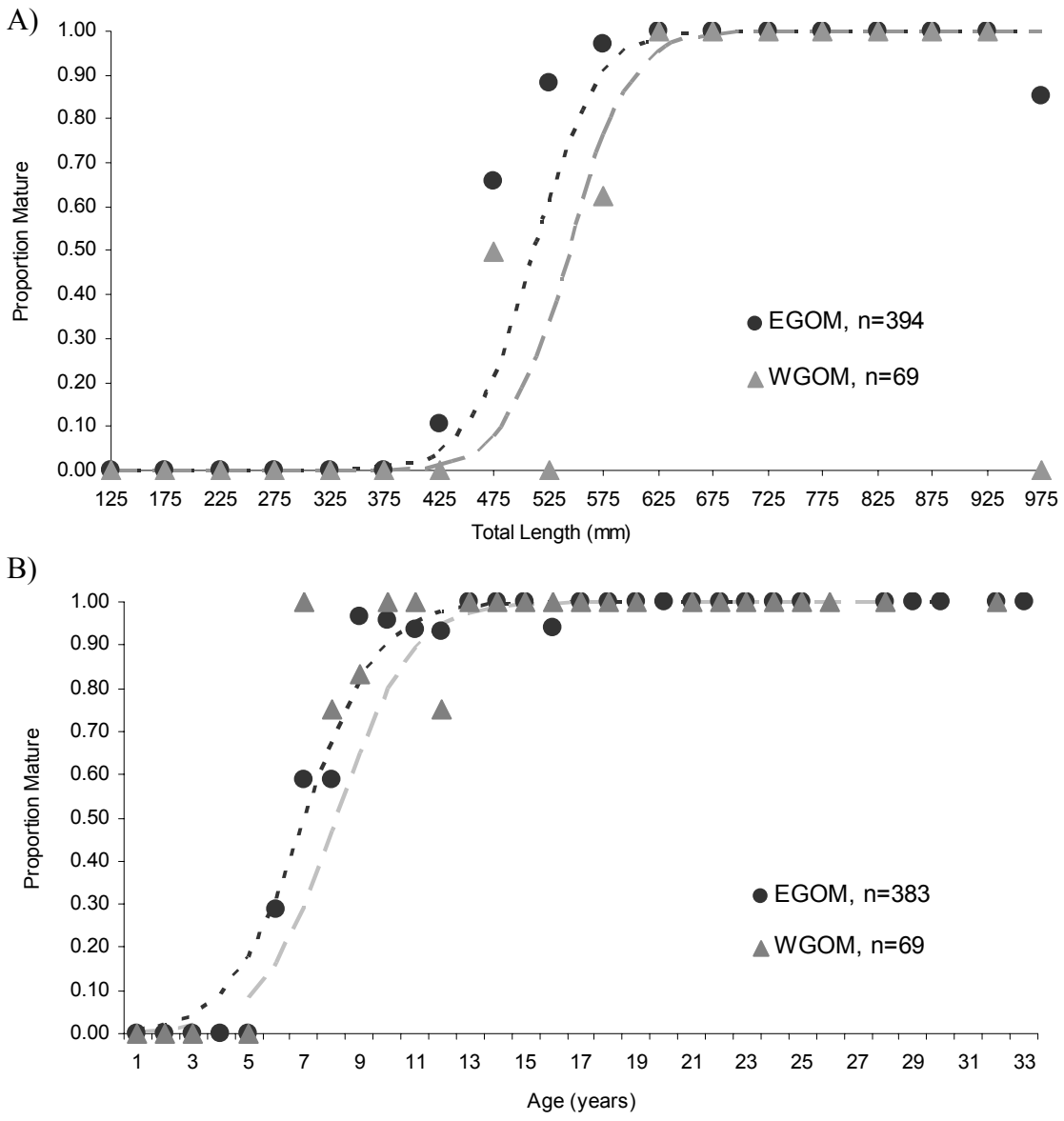

Figure 21. Maturity estimates calculated for A) total length and B) age at 50% mature for female yellowedge grouper collected in the eastern (E) and western (W) Gulf of Mexico (GOM). Total length represents the midpoint of each size class.

Females continued to reproduce throughout their lifespan as older individuals (>30 years old) were spawning-capable. By 23 years (95% CI=19-27) of age and 840 mm TL (95% CI=714-966) 50% of females in the population had transformed into males (Figure 22 and 23 A, B). Differences were also observed between the eastern GOM and western GOM, however, overlapping confidence intervals indicated that observed differences were not conclusive. Size and age at 50% sexual transition in the eastern GOM was 811 mm TL (95% CI=664-992) and 22.0 years (95% CI=18-28) while size and age was larger in the western GOM, 865 mm (95% CI=595-1,660) and 24.8 years (95% CI=17-46), respectively (Figure 24 A, B).

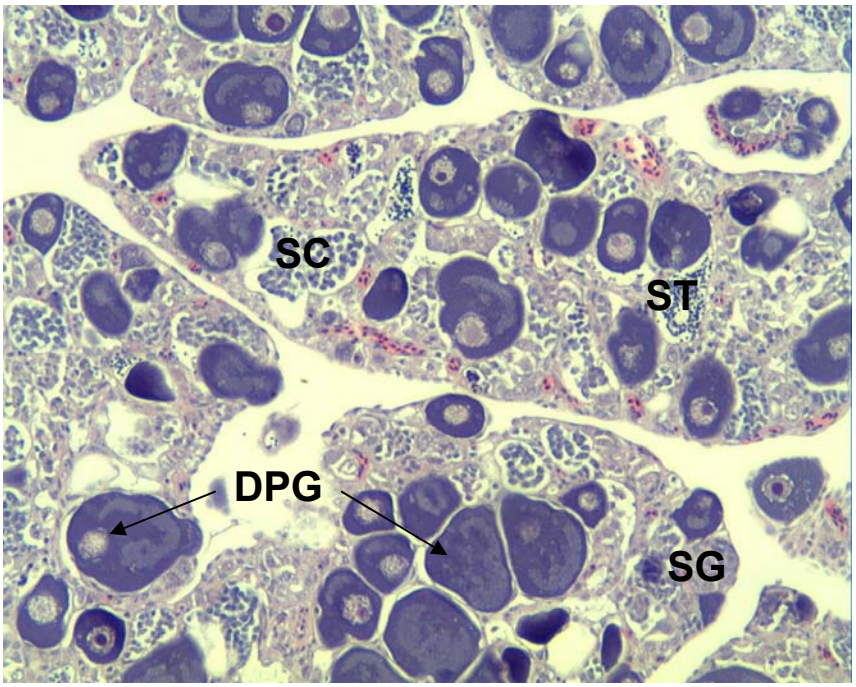

Figure 22. Histological section of a yellowedge grouper gonad undergoing sexual transition. Primary growth oocytes are degenerating (DPG) and spermatogenic tissue is proliferating. All stages of spermatogenesis present including spermatogonia (SG), spermatocytes (SC) and spermatids (ST). Photograph taken at 80x magnification.

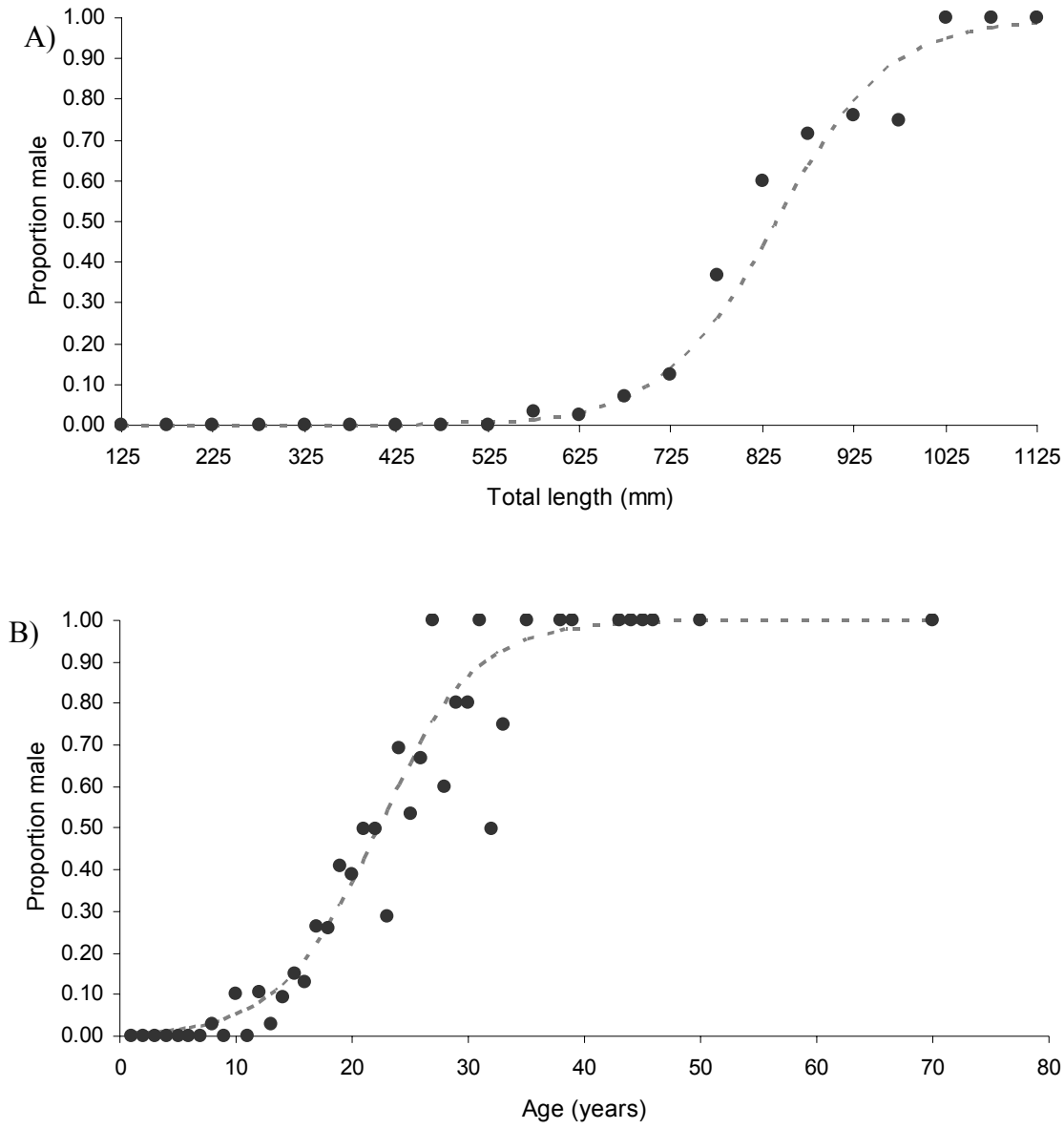

Figure 23. A) Total length and B) age at which 50% of female (*n*=468) yellowedge grouper have transformed into males (*n*=134). Total length represents the midpoint of each size class.

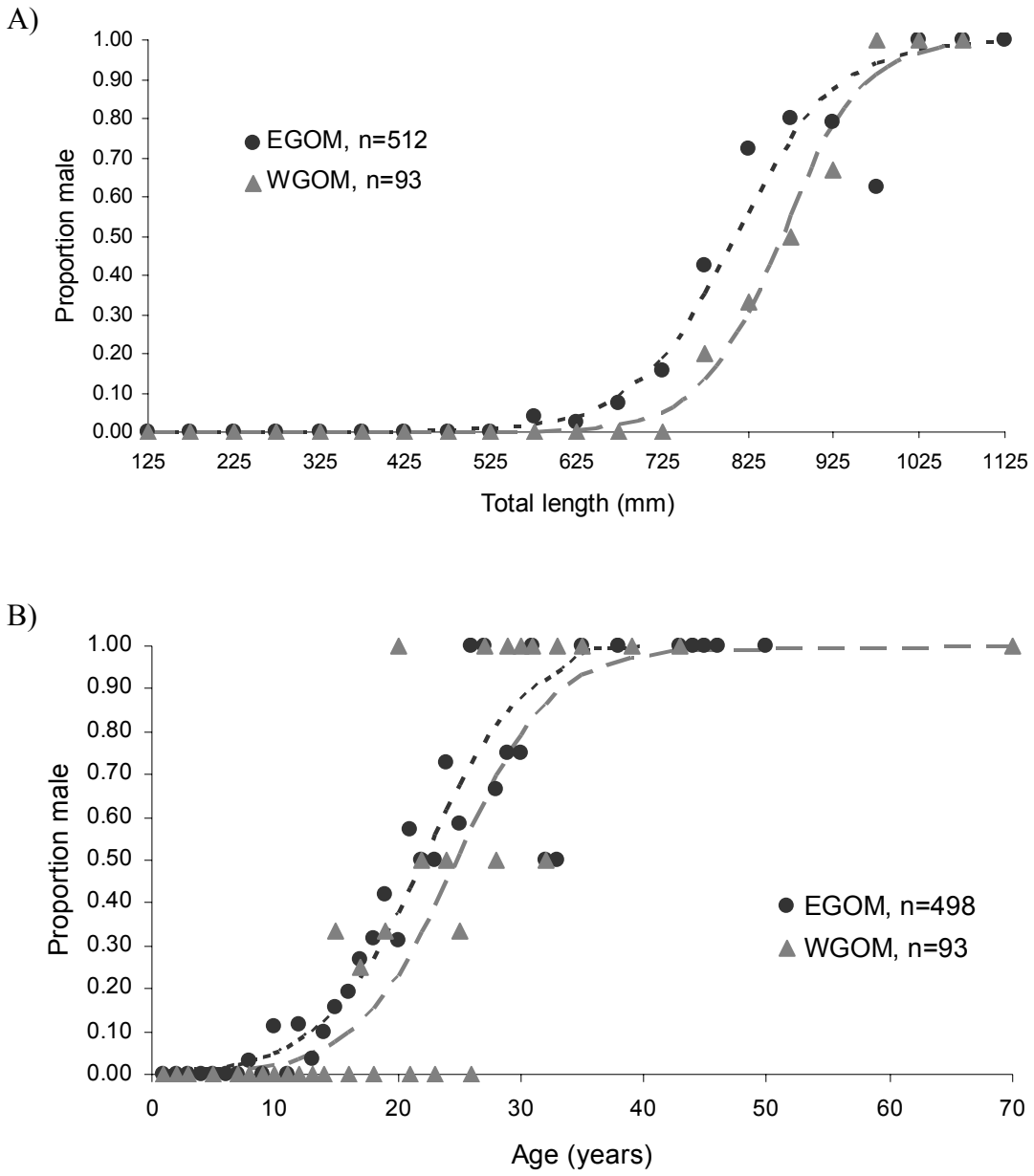

Figure 24. A) Total length and B) age at which 50% of female yellowedge grouper from the eastern (E) and western (W) Gulf of Mexico (GOM) transformed into males. Total length represents the midpoint of each size class. Sample size from the eastern GOM: females *n*=398, males *n*=114 and western GOM: females *n*=70, males *n*=23.

Histological examination of testes indicated that all males collected were sexually mature. Regardless of month of capture all testes contained spermatozoa in the ducts. Primary spermatogonia were present in the testes in February through September. Males classified as regenerating were the only sexual maturation class (Figure 25) not encountered. Many of the testes contained remnant primary growth oocytes indicating that they may have recently changed sex or that primary growth oocytes take longer to resorb than other oocytes. The physical structure of the testes visually resembled that of ovaries and contained lamellae like framework which is consistent of protogynous hermaphrodites. This occurred in all yellowedge grouper with the exception of one male which resembled that of a gonochoristic male (Figure 26). The potential primary male was 17 years old and 756 mm TL. Primary males have not been reported in yellowedge grouper. Numerous macrophage aggregates were observed in all of the testes, with the exception of the potential primary male.

Sex Ratio. The overall sex ratio for yellowedge grouper sampled was 1:3.5 (male:female), which is significantly different from 1:1 (X^2=185.309, P<0.0001). The sex ratio of yellowedge grouper from the eastern GOM (n=512) and western GOM (n=93), 1:3.6 and 1:3.0, respectively, was not significantly different (X^2=2.67, P=0.101). A significant difference was found between the sex ratio of yellowedge grouper sampled from 1977-1980, 1:1.8 (male:female), (Bullock et al., 1996) and yellowedge grouper sampled during 1999-2005 (X^2=51.86, P<0.0001). A significant difference was also found between the sex ratio of yellowedge grouper sampled from the eastern GOM during 1977-1980 (Bullock et al., 1996) and yellowedge grouper sampled from the eastern GOM (1:3.6) (X^2=46.501, P<0.0001) during this study.

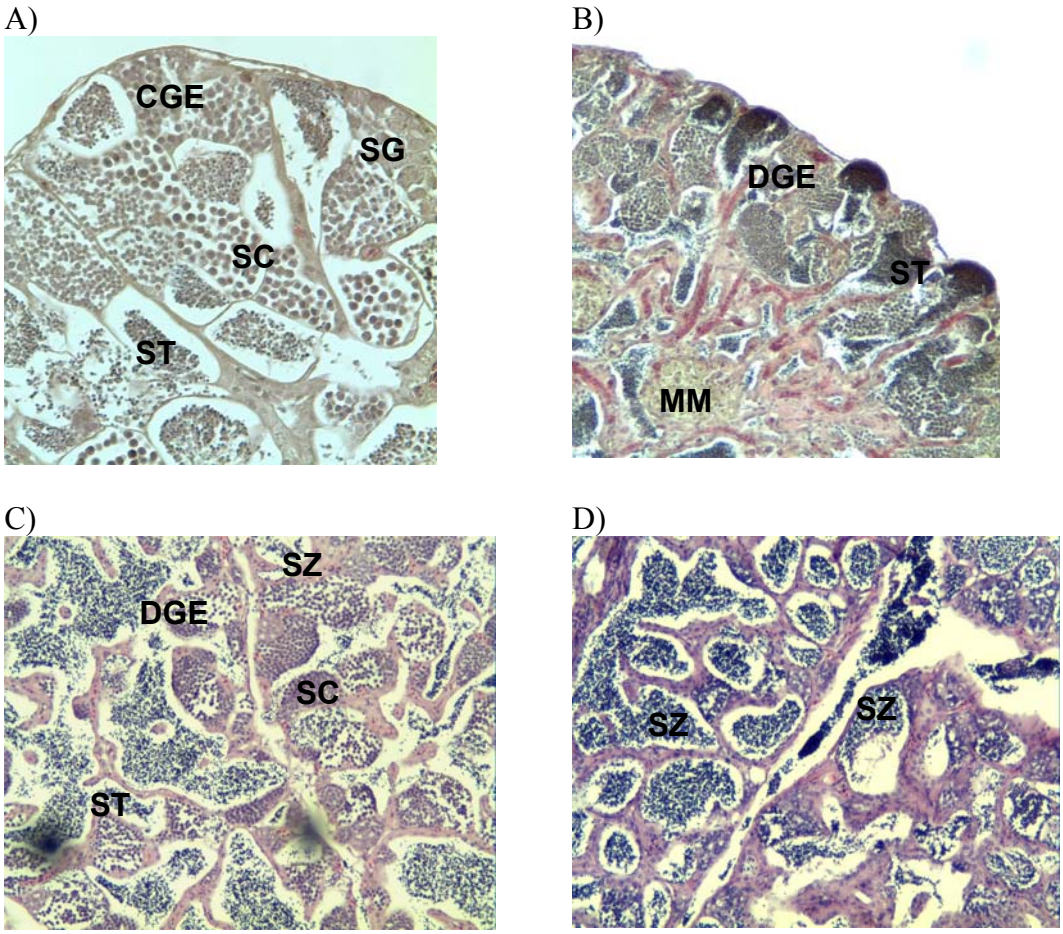

Figure 25. Histological sections of testicular tissue from yellowedge grouper in various maturation classes. A) Early maturation, all lobules have a continuous germinal epithelium (CGE). B) Mid-maturation, lobules near the periphery have a discontinuous germinal epithelium (DGE). C) Late maturation, discontinuous germinal epithelium at ducts and periphery. D) Regressing, reduced spermatogenesis, extensive amounts of spermatozoa (SZ), lobules begin to fuse. Spermatogonia (SG), spermatocytes (SC), spermatids (ST) and melanomacrophages (MM) may also be present depending on stage. All photographs taken at 200x magnification.

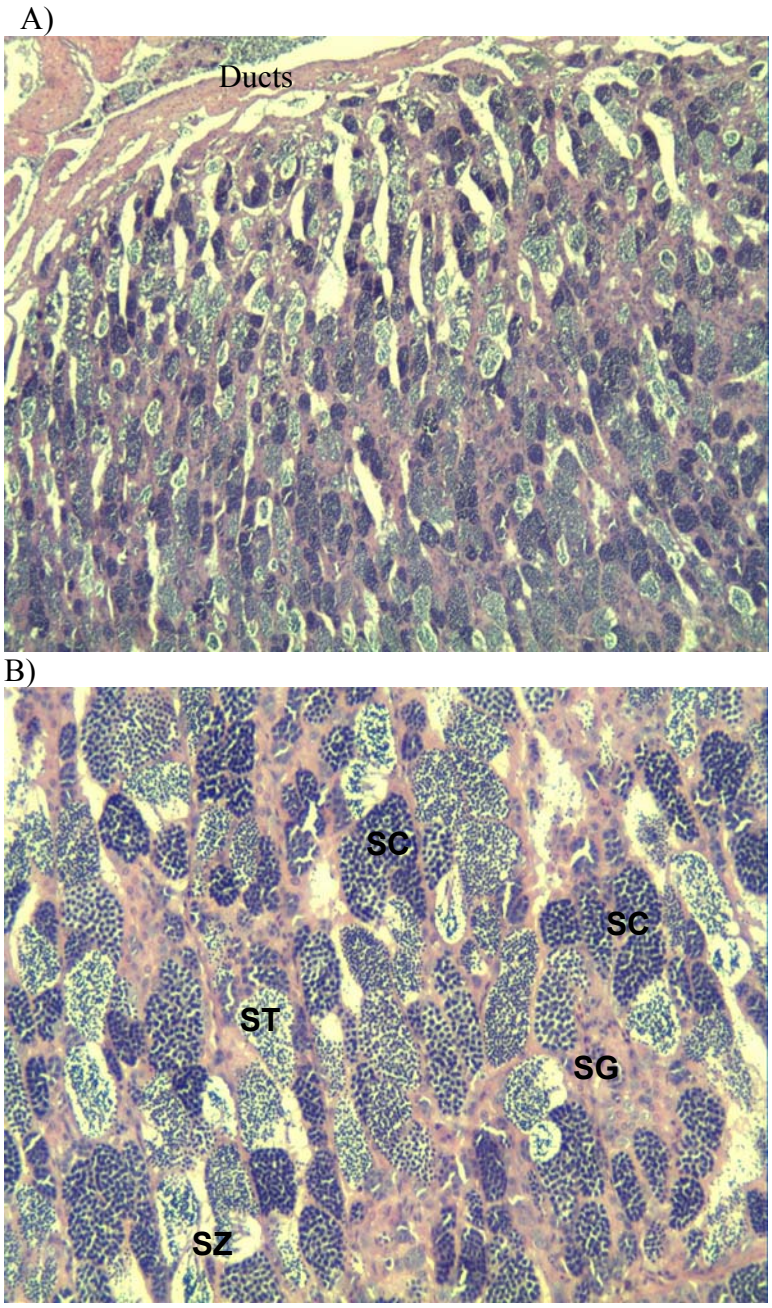

Figure 26. Histological sections of the testes of a potential primary male yellowedge grouper. A) Duct area of the testes taken at 80x magnification. B) Lobules taken at 200x magnification. Active spermatogenesis observed throughout the gonad. Spermatogonia (SG), spermatocytes (SC), spermatids (ST) and spermatozoa (SZ) present.

Spawning Season. Regression analysis indicated a significant relationship between female GSI and gonad-free body weight when females of all reproductive classes were combined (n=444, r^2=0.140, P<0.000). However, regression analysis of females excluding immature and regenerating individuals (n=291, r^2=0.032, P=0.002) indicated that GSI could be used to predict the reproductive season. Although a significant relationship was detected between gonad-free body weight of active females and GSI it was probably not biologically relevant since the regression only explained 3.2% of the variation in the data. A significant relationship was detected for immature and regenerating females (n=148, r^2=0.218, P<0.000) indicating that GSI is closely associated with fish size in inactive females. A significant difference (ANOVA, $F_{7,283}$=11.78, P<0.001) occurred between monthly mean female GSI values (Figure 27). GSI values were the highest in July through September which coincided with the highest percent of females in FOM. Only February and July mean monthly GSI values differed between males (ANOVA, $F_{7,121}$=3.29, P=0.003; Bonferroni, P=0.014) indicating no obvious pattern in male GSI values.

Females were collected in all sampling months while males were collected in all months except October and December. Transitional fish were only collected in April and May, prior to the peak in spawning season. Transitional fish made up <1% of fish sampled indicating that transition occurs quickly. Actively spawning females (n=34) were collected from March through September (Figure 28) but were most abundant in August and September. Regressing females (n=17) were collected in June through September. Regenerating females (n=91) were most abundant in December and February but were collected during most of the year, indicating that although spawning occurs for

seven months individual fish may not spawn for the entire season. There was no significant difference (ANOVA, $P>0.05$) between the size or age of regenerating or FOM fish on a monthly basis indicating that neither influenced the time of spawning. All males collected contained spermatozoa and were capable of spawning.

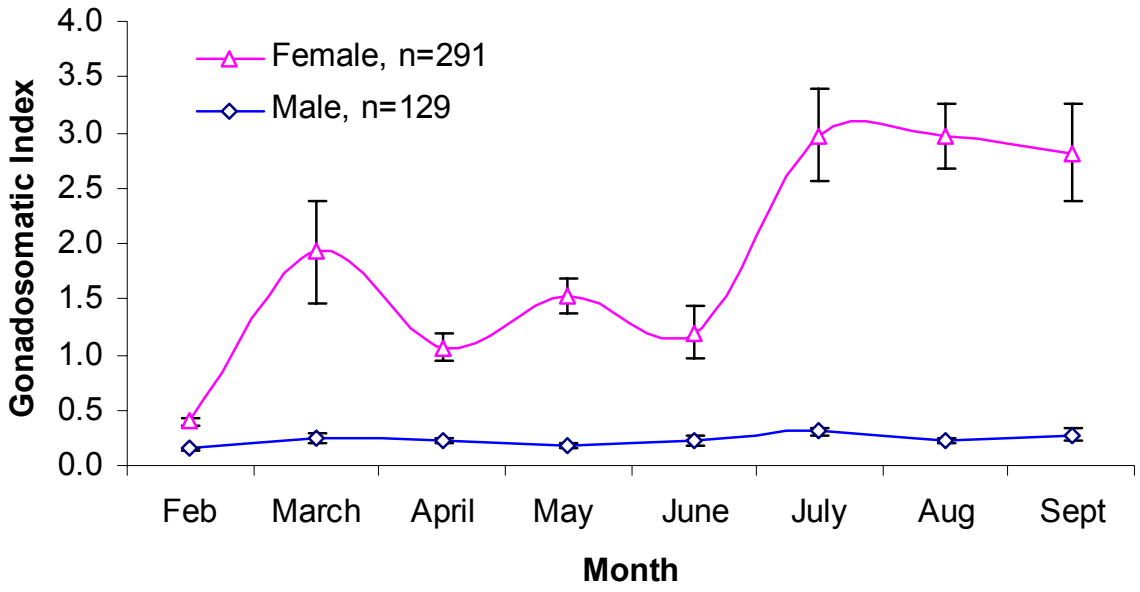

Figure 27. Yellowedge grouper monthly mean Gonadosomatic Index (GSI) values. Error bars represent ±1 standard error, sample sizes are presented for each month, immature and regenerating reproductive classes excluded.

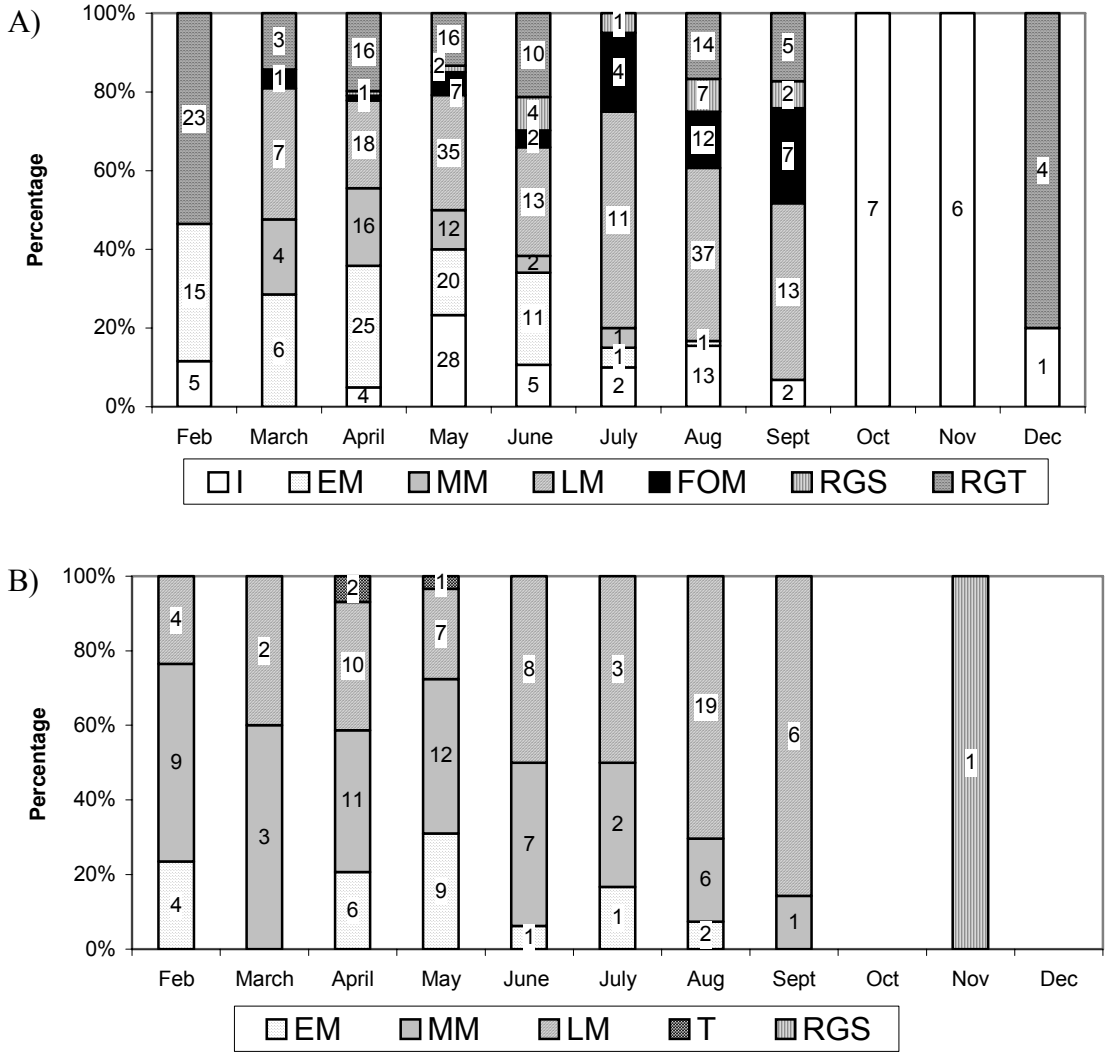

Figure 28. Monthly percentages of reproductive class of A) female (*n*=463) and B) male (*n*=134) and transitional (*n*=3) yellowedge grouper collected by fishery dependent and independent sources during 1999-2005. Values represent the number of fish in reproductive class. Reproductive classes are defined as follows, I=immature, EM=early maturation, MM=mid-maturation, LM=late maturation, FOM=final oocyte maturation, RGS=regressing, RGT=regenerating, and T=transitional male.

Spawning frequency. Sampling occurred 24 hours per day on MS Lab surveys, however, few hydrated females (n=8) were captured. Hydration occurred in the early morning through early afternoon hours (Figure 29). Females undergoing FOM, which contained migratory nucleus oocytes and oocytes undergoing lipid coalescence (n=26), were collected between 0400 to 2200 hours central standard time with the majority collected in the morning. These data suggest spawning probably occurred during the morning to late afternoon hours in depths of 84 to 284 m (mean=196 m). Bottom water temperatures ranged from 11 to 20°C (mean=16°C).

Spawning frequency varied significantly by month (FOM, X^2=20.252, P<0.0001; POF, X^2=17.789, P<0.0001) and method of calculation (Table 15). Monthly spawning frequency was calculated for the duration of the spawning season (March through September) adjacent months in which <25 spawning-capable fish were collected were combined with the exception of September which yielded twenty samples. Since only five fish with POF classified as less than 24 hours old were collected the FOM method produced more accurate spawning frequency results. The overall spawning frequency based on the FOM method averaged every 4.8 days. Yellowedge grouper spawned less frequently in the beginning of the season (every 13.5 days) and spawned every 2.9 to 4.0 days by the peak of the reproductive season. Yellowedge grouper spawned on average every 25.7 days based on the POF method. Only results from September were comparable using both methods, however, sample size for September was smaller than that of other months. Yellowedge grouper spawned every 2.9 days and 6.8 days in September based on the FOM and POF methods, respectively.

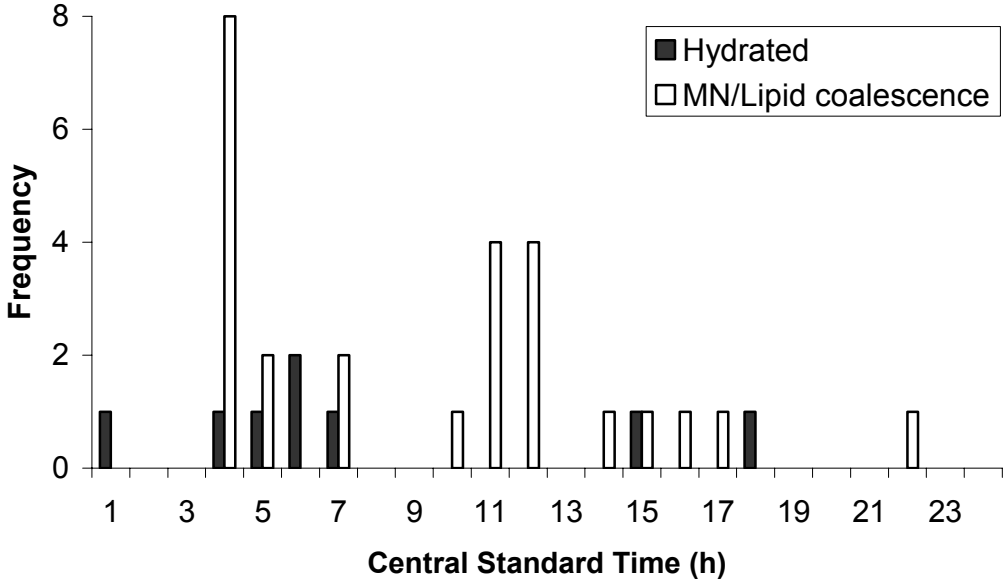

Figure 29. Time of spawning of yellowedge grouper collected by fishery dependent and independent sources from 1999-2005. Hydrated females (*n*=8) and females undergoing migratory nucleus (MN) and lipid coalescence (*n*=26) were sampled over a 24 hour time period. Time is recorded as the hour the set began. Soak times varied from 1-7.7 hours (mean= 3.2 hours).

Table 15. Monthly spawning frequency. Yellowedge grouper spawning frequency was calculated using the number of females experiencing final oocyte maturation (FOM) and the number of females with 0-24 hour postovulatory follicles (POF). Yellowedge grouper were considered spawning-capable if oocytes were in the late maturation or FOM class. **Mean spawning frequency (days).

	Month(s)					Total
	March/April	May	June/July	August	September	Mean**
Number Spawning-capable	27	42	30	49	20	168
Number FOM	2	7	6	12	7	34
Number 0-24 hr POF	0	1	0	1	3	5
Days in time period	61	31	61	31	30	214
Spawning frequency FOM	13.50	6.00	5.00	4.08	2.86	4.83**
Spawning frequency POF	0.00	42.00	0.00	49.00	6.67	25.74**

Due to the small sample size and lack of documentation describing the longevity of yellowedge grouper POF, the FOM method appeared to more accurately illustrate spawning frequency. Annual spawning frequency averaged 44 times per year. A conservative estimate of spawning frequency using 13.5 days between spawning events yielded 16 times per year, conversely if yellowedge grouper spawned every 2.9 days annual spawning frequency is 75 times per year. Average annual spawning frequency using the POF method was eight times per year (range 5-32 times).

Gonad weight proxy for fecundity. The relationship of gonad weight of females (with vitellogenic or hydrated ova) verses total length (Figures 30 A), whole weight (B) and age (C) were used as a proxy for fecundity. The relationship between gonad weight and whole weight appeared to best represent the data (R^2=0.448) followed by total length (R^2=0.416) and lastly age (R^2=0.225).

It was observed that not all unshed hydrated eggs were resorbed annually by yellowedge grouper. Five percent of all yellowedge grouper gonads sampled contained hardened masses of compacted eggs in the lumen. This occurred in both females (3.2%; Figure 31 A) and males (11.2%; B)). Females ranged in age from 9-22 years old and males were 10-45 years old suggesting that once these masses form they may never fully resorb. Fish health and reproductive development did not appear to be affected since masses were found in females in all reproductive classes except FOM (possibly due to lack of samples) and males in all classes. It is unknown if the masses impact spawning capability, however, in some fish the mass occupied a large portion of the gonad.

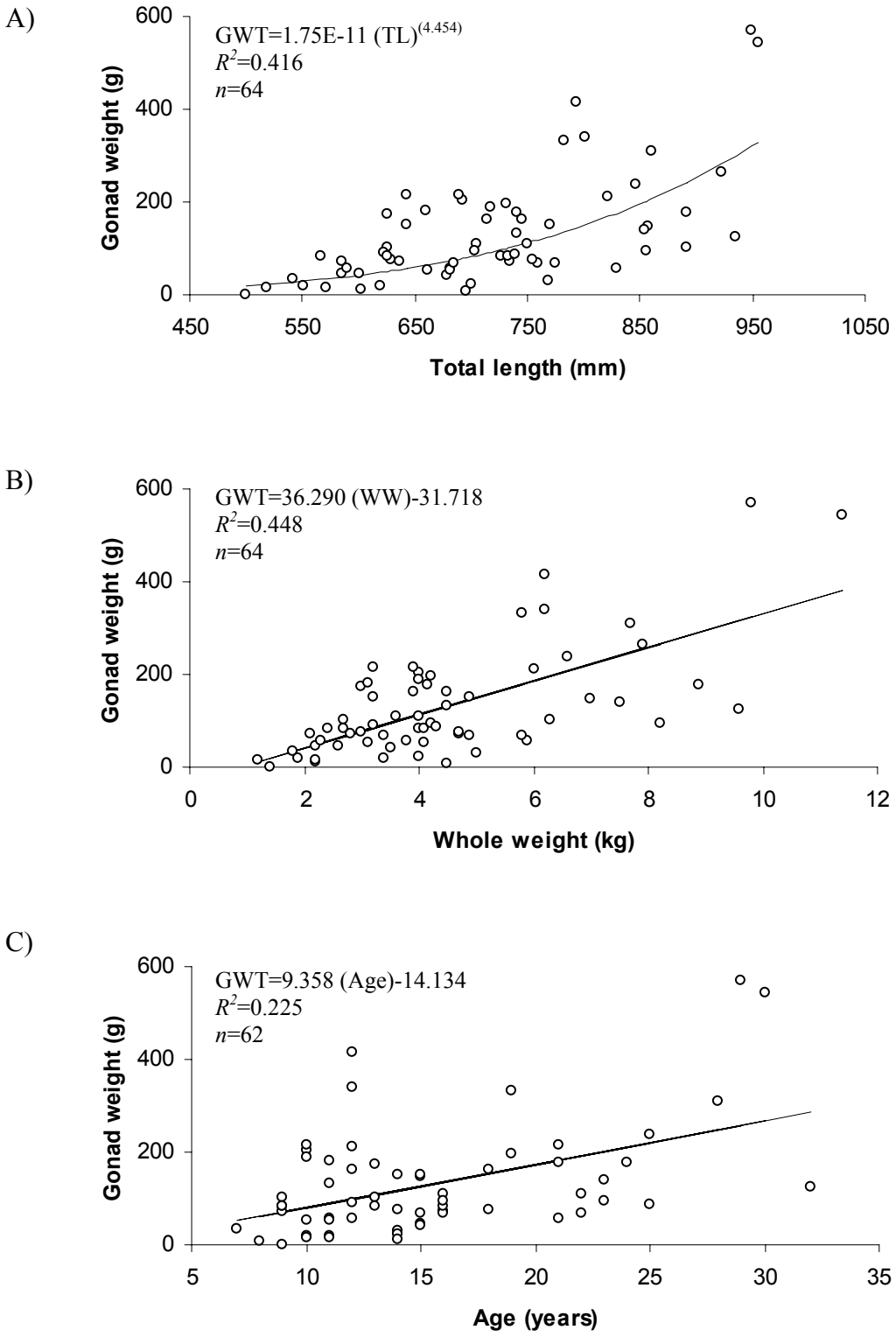

Figure 30. Gonad weight (GWT) by A) total length (TL), B) whole weight (WW) and C) age of female yellowedge grouper with vitellogenic or hydrated ova. Regressing stages excluded.

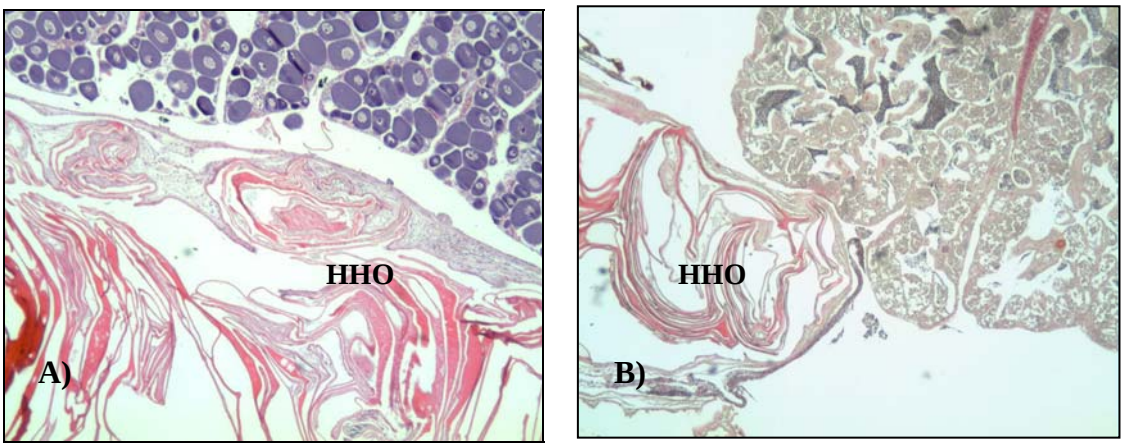

Figure 31. Hardened mass of degenerated hydrated oocytes (HHO) in a A) regenerating female and B) mid-maturation male. Photographs taken at 80x magnification.

Discussion

Most grouper are long-lived, slow growing and many species can reach maximum ages of 30-40 years (Moe, 1969; Collins et al., 1987; Crabtree and Bullock, 1998; Wyanski et al., 2000). Scamp, *Mycteroperca phenax*, have been reported to reach ages of 30 years in the South Atlantic Bight (Harris et al., 2002). Black grouper, *Mycteroperca bonaci*, from the Florida west coast and Keys reached ages of 33 years (Crabtree and Bullock, 1998). Warsaw grouper (*Epinephelus nigritus*) and goliath grouper (*Epinephelus itajara*) were reported to be the oldest grouper reaching ages of 41 and 37 years, respectively (Bullock et al., 1992; Manooch and Mason, 1987). Current results found that yellowedge grouper can live in excess of 85 years suggesting they may have the longest lifespan of any grouper found in the GOM.

^{14}C analysis established that yellowedge grouper longevities exceeded previously published estimates of 15 years (Keener, 1984) and 35 years (Manickchand-Heileman and Phillip, 2000). The ^{14}C core analysis demonstrated that yellowedge grouper reach at

least 41 years of age by back-casting from the most recent date of capture. Yellowedge grouper captured in 2001 with negative $\Delta^{14}C$ levels (n=9) were at least 41 years of age because only positive $\Delta^{14}C$ levels have been detected since 1960. Therefore, a negative $\Delta^{14}C$ level estimated a birth date of at least 1960 resulting in a minimum age of 41 years. By plotting the otolith estimated age verses the level of $\Delta^{14}C$ found in the otolith it was possible to view the changes in $\Delta^{14}C$ over time.

The ^{14}C chronology for yellowedge grouper was comparable to that of other marine organisms. The similarities between the yellowedge grouper $\Delta^{14}C$ curve and red snapper (Baker and Wilson, 2001) and coral (Druffel, 1980; Druffel, 1989) $\Delta^{14}C$ chronologies indicated that yellowedge grouper were also influenced by the nuclear-bomb produced radiocarbon. If otolith age estimates based on growth increment counts were incorrect, the yellowedge grouper $\Delta^{14}C$ curve would have been skewed from the other curves. This supported the hypothesis that growth increments are laid down annually and otolith-based ages are valid estimates of yellowedge grouper age. Comparison of coral and red snapper $\Delta^{14}C$ values and yellowedge grouper $\Delta^{14}C$ values was appropriate because the chronologies from yellowedge grouper, GOM red snapper (Baker and Wilson, 2001) and corals collected in Bermuda (Druffel, 1989), south Florida (Druffel, 1989) and Belize (Druffel, 1980) were not significantly different.

The majority of yellowedge grouper $\Delta^{14}C$ values closely resembled those found in published chronologies; however, the $\Delta^{14}C$ levels of some otolith cores deviated from the chronology. Sample #333 had a slightly higher $\Delta^{14}C$ value (-22.1‰) than would be expected for a fish born in 1939. This fish was one of the first 11 samples submitted for AMS analysis, and may have contained additional otolith bands from the distal area

below the core. If so, this could have contributed to elevated $\Delta^{14}C$ levels. The method of core extraction was modified from dental instrument extraction to Dremel extraction to allow for a more homogenous sample and improved isolation of the core. Since no significant difference was found between the submitted cores and blind duplicates this modification appeared to eliminate the problem. Also, samples 1423, 1434, 1442 and 1471 had $\Delta^{14}C$ values that deviated slightly from the curve with lower than expected $\Delta^{14}C$ values. Samples 1434 and 1471 were from the 1974 cohort and had the same $\Delta^{14}C$ value of 112.3 ‰; the fish were collected in Texas waters 17 years apart. This suggests that something, such as unusual environmental or oceanographic conditions, may have occurred during 1974 to influence ^{14}C levels in the Gulf (those are the only records from 1974) or in the region where those juvenile yellowedge grouper were located.

Levels of ^{14}C are directly influenced by the levels of dissolved inorganic carbon (DIC) in the surrounding water column. Approximately 70% of the carbon used in otolith formation is derived from DIC which is supplied to the inner ear during the assimilation of seawater through the gills. The remaining 30% of carbon is derived from ingested food (Degens et al., 1969; Kalish, 1991 a, b). If the DIC or prey items had lower levels of ^{14}C than would be expected for those time periods it could have influenced the levels of ^{14}C in the otoliths. Several cores from fish born before 1960 (n=9) had $\Delta^{14}C$ levels below the expected range of values (-51‰ to -62‰), which possibly indicated the Suess effect. Suess (1955) demonstrated that the burning of fossil fuels after 1900 resulted in the release of ^{14}C-free CO_2 which diluted atmospheric and oceanic radiocarbon between 1900 and the onset of nuclear-bomb produced radiocarbon causing lower than expected ^{14}C levels.

My study is the first to apply multiple section analysis to an otolith. Results indicated that yellowedge grouper can reach ages of 85 years. Although the analysis cannot identify specific years as the core analysis can it does support an age estimate of 85 years based on the numerous sections containing negative $\Delta^{14}C$ values on a single otolith. As a fish grows, calcium carbonate is deposited on the otolith. Growth is faster initially and then slows as the fish ages resulting in less calcium carbonate deposition. The isolation of growth increments with negative $\Delta^{14}C$ values in multiple areas on a single otolith suggested that those areas were deposited before the 1960's increase in ^{14}C. A disadvantage of the multiple section analysis was that samples were required to contain numerous years to allow for enough material for ^{14}C analysis and the amount of otolith sampled each year was unknown. Therefore, the reported $\Delta^{14}C$ values represented the sum of a combination of years instead of a single year estimate since the quantity of ^{14}C absorbed each year was unknown. The isolated section analysis was used as a tool to verify time periods (pre-bomb or post-bomb) on a single otolith.

The time period of formation was classified correctly for most otoliths, however, there were two exceptions. Samples 101 Z and 283 Z were both classified as post-bomb sections based on estimated age but exhibited negative $\Delta^{14}C$ values of -37.1 and -39.9‰, respectively. These sections contained growth increments deposited during the early 1960's when ^{14}C levels were beginning to increase but were still negative values (<0‰). Although the multiple section analysis was not as precise as the core section analysis it was a suitable way to attempt to validate ages in excess of 41 years; providing a useful new method to validate age estimates of long-lived fishes.

The longevity of yellowedge grouper compared to many other grouper in the

GOM may be due to their deep-water habitat preference and related physiological processes which allow for adaptation to harsh environmental conditions such as low light, oxygen, temperature and prey availability associated with the deep ocean (Cailliet, 2001). Cailliet et al. (2001) presented evidence that deep-water species may live considerably longer than shallow-water species and found an exponential relationship between maximum-recorded depth and maximum age estimates for 47 species of *Sebastes*. Numerous deep-water marine species with validated age estimates have long lifespans. Some elasmobranchs reach ages of 50-75 years, grenadiers can live 50-70 years, several deep water seamount fishes live over 100 years and rockfishes reach ages of 60-205 years depending on species (Cailliet et al., 2001). Little information is available regarding maximum age estimates for most deep-water groupers in the GOM; however, they may follow the same pattern as yellowedge grouper.

Limited life history information on yellowedge grouper was available prior to this study. Yellowedge grouper age and growth research was conducted by Keener (1984) in South Carolina and Bullock et al. (1996) in western Florida. Keener (1984) processed 590 sagittal otoliths; however, she was only able to age 27% of her samples. Ages were estimated for only those otoliths with readily distinguishable annuli. Age estimates for the South Carolina commercial fishery ranged from 2-15 years, however, due to the uncertainty of assigning ages to larger fish Keener (1984) estimated that ages could exceed 20 years. Bullock et al. (1996) considered most yellowedge grouper otoliths unreadable; therefore, ageing attempts were unsuccessful. Bullock et al. (1996) and Keener (1984) independently concluded that yellowedge grouper otoliths were difficult to read because opaque growth increments were not easily distinguishable. Additional

ageing research was conducted in Trinidad and Tobago with greater success (Manickchand-Heileman and Phillip, 2000). Yellowedge grouper were reported as long-lived, reaching ages up to 35 years. Manickchand-Heileman and Phillip (2000) examined 367 sagittal otoliths and were able to count annuli on 89% (*n*=326) of their samples. A subsample of 63 otoliths independently read by two readers resulted in 95% reader agreement.

Yellowedge grouper otolith age analysis was successful in this study although ageing difficulties were encountered. While most otoliths were classified as readable they were considerably more challenging to age than other grouper found in the GOM and Atlantic (Moe, 1969; Collins et al., 1987; Crabtree and Bullock, 1998; Wyanski et al., 2000). The otoliths of small yellowedge grouper were as challenging to age as those of larger fish. The first two growth increments were fairly easy to interpret while the next several years were often difficult to determine. Growth increments beyond 15-20 years appeared to layer above each other but could often be distinguished. The most difficult section of the otolith to interpret was the edge since it often appeared very dark in color.

Only 13% of otoliths were aged identically by both readers supporting the difficulty observed by previous investigators. Reader precision for long-lived species tends to vary considerably more than for fish with only a few age classes. Beamish and Fournier (1981) noted that a 95% age agreement within ±5 years for spiny dogfish (*Squalus acanthias*) can represent very good precision since dogfish can live up to 60 years. This also seemed to be the case regarding yellowedge grouper reader precision which indicated that 78.5% of otoliths could be aged within ±5 years. Reader

comparisons did indicate that age estimates were more precise over time as readers gained experience, however, yellowedge grouper APE (10.0%) may never reach that of easier to age species such as red snapper (APE=2.5-6.0%; Allman et al., 2005a) or red grouper, *Epinephelus morio*, (APE=3.5%, CV=4.3%; Palmer et al., 2006). The strong relationship between otolith weight and fish age could be used by future investigators to confirm the accuracy of ages determined by viewing otoliths. In situations where time constraints exist it may be appropriate to use otolith weight as a means to predict final age. Due to reader uncertainty and lack of precision it is most likely impossible to monitor year classes and year class strength over time. Age estimates are accurate enough to follow the general trends of the population but the patterns of a specific cohort could be lost due to over or under estimating the age of individual fish.

Ageing attempts by Bullock et al. (1996) and Keener (1984) possibly resulted in low ages because readers had difficulty determining individual growth increments. Bullock et al. (1996) viewed the otoliths using polarized light which probably did not provide enough light to distinguish individual growth increments. Keener (1984) used reflected light, which tends to wash out the growth increments and make them difficult to distinguish. Previous Gulf of Mexico and Atlantic studies found most grouper reached ages of 20-41 years (Moe, 1969; Collins et al., 1987; Manooch and Mason, 1987; Bullock et al., 1992; Crabtree and Bullock, 1998; Wyanski et al., 2000) depending on species. Since grouper were not expected to live much beyond 40 years it is possible that Bullock et al. (1996) and Keener (1984) did not consider each of the increments on the yellowedge grouper otoliths to be annual marks. Yellowedge grouper in the GOM were found to be considerably older than those in Trinidad and Tobago (Manickchand-

Heileman and Phillip, 2000). Although genetic information is currently lacking, Caribbean yellowedge grouper may be from separate populations and experience considerably different levels of fishing pressure which could influence the age structure of the stock. Manickchand-Heileman and Phillip (2000) also had a small sample size (n=367) which may have reduced their chances of detecting the oldest fish in their region.

Ageing difficulty is not a problem experienced only in yellowedge grouper research. Rhodes and Sadovy (2002) reported poor legibility of sagittal otoliths from camouflage grouper, *Epinephelus polyphekadion*, and were unable to report age estimates. Harris et al. (2002) were able to only age 82% of scamp otoliths. Over 565 otoliths were discarded because age agreement could not be reached by two readers. Crabtree and Bullock (1998) reported difficulty estimating ages of black grouper older than 10 years because growth increments narrowed as fish aged.

The ages of yellowedge grouper collected by fishery dependent and independent sectors were similar, probably because the majority of yellowedge grouper captured by each sector were collected using bottom longline gear. The gear and methods used in fishery independent surveys were similar to those of the commercial fishery. However significant age differences between bottom longlines, hand lines and other gear types were observed. Selectivity between gear types is common and can influence the size of fish captured. Cass-Calay and Bahnick (2002) reported that commercial bottom longlines captured larger yellowedge grouper than commercial hand lines. Most of the yellowedge grouper collected by "other" gear types were captured using trawls which typically capture smaller fish than longline gear or hand line gear (Roe, 1976; Lokkeborg and Bjordal, 1992; Clarke et al., 2002).

Small sample size during early time periods and gear bias probably contributed to the lack of small fish collected and perhaps influenced the significant differences found between frequency distributions over time. Typically mean or median fish size decreases over time as a result of fishing pressure as larger fish are often removed first from the population (Bullock et al., 1996; Coleman et al., 2000). However, my results indicated that yellowedge grouper were larger during 1991-1992 and smallest during 1979-1989 when fishing pressure was presumably less. Only 148 fish were available during 1979-1989, of which 35% were collected using hand line gear, verses 367 fish during 1991-1992 and 1,172 yellowedge grouper collected during 1999-2005. Results of the length-frequency and age-frequency analyses appear to be confounded by gear bias and small sample size during the earlier time periods.

Bullock et al. (1996) noted a significant change in the length-frequency distributions of yellowedge grouper collected between 1977-1980 and 1993-1994, fish were smaller during 1993-1994 (mean=650 mm TL). My results indicated that median size of yellowedge grouper collected during 1991-1992 was slightly larger in size than fish collected during 1993-1994 (Bullock et al., 1996). This could indicate that a decrease in size occurred in just a few years or the size differences observed between the eastern and western GOM may have contributed to these findings. All fish collected during 1991-1992 were from the western GOM which appears to currently have a larger size at age than yellowedge grouper from the eastern GOM. Bullock et al. (1996) only collected fish from the eastern GOM; therefore, the smaller size may be due to differences between the populations. Unfortunately a lack of samples collected during 1991-1992 from both regions prevented a direct comparison during that time. Results

from yellowedge grouper collected during 1999-2005 indicated that the length-frequency distribution of yellowedge grouper has changed little since the early 1990's.

Comparisons using only bottom longline gear appear a more appropriate method to view changes in fish size over time. The mean size of yellowedge grouper collected by Bullock et al., (1996) during 1977-1980 was 758 mm TL (n=3,577) which is considerably larger than fish collected during 1979-1989 using all gear types, however, similar in size to yellowedge grouper collected using only bottom longline gear (mean=731.4 mm TL) during that time. Although Bullock et al. (1996) did not report the number of fish collected by gear type, the majority of grouper harvest in the eastern GOM during that time occurred using bottom longline gear (Prytherch, 1983). The lack of difference observed between 1991-1992 and 1999-2005 supported the results of the length-frequency analysis.

Bullock et al. (1996) were unable to provide age estimates for their samples so it was not possible to compare the two studies. Comparison of the age-frequency distributions over time indicated no significant change in age structure. However, the change in age structure was apparent when examining only bottom longline data. Yellowedge grouper were considerably older during 1979-1989 than in the remaining time periods despite the small sample size and large number of years included during that time period. Fish aged during 1979-1989 were an average ten years older than those from remaining time periods. A considerable decrease in the number of older fish present in the population was also observed. Although only a slight drop in the percent of older fish occurred between 1979-1989 and 1991-1992, the change was significant in recent fish collected. Currently only an estimated 5% of yellowedge grouper were older

than 34 years old as opposed to 47 years old observed during 1991-1992. Although average fish size did not change between 1991-1992 and 1999-2005 it is apparent that a considerable difference in the age structure of the populations has occurred over the last 25 years.

Although mean size and age structure have decreased since the onset of fishing, changes in growth rate over time were not apparent despite significantly different von Bertalanffy growth curves. Parameters L_∞ and K are highly correlated and sensitive to the range and distributions of observations used in the analysis (Newman et al., 1996). Considerably smaller fish were collected during 1999-2005 than in other time periods. Comparisons between von Bertalanffy growth curves are influenced by sample size (Cerrato,1990). Comparable sample sizes were not available over time or between regions and possibly influenced results. The von Bertalanffy growth curve is also influenced by the number of young fish in the sample which drives t_0 and the maximum age of the fish (Haddon, 2001). Yellowedge grouper of ages zero and one were only collected during 1999-2005. The youngest fish collected in 1979-1989 and 1991-1992 were four and two, respectively. The lack of young fish during some years and not during others probably resulted in the significant difference observed between growth curves.

Growth curve comparisons of recently collected yellowedge grouper from the eastern and western GOM indicated significant differences in growth between the two regions. Faster growth occurred in the western GOM supporting the larger size at age observations and the hypothesis that more than one population of yellowedge grouper occurs in the GOM. If yellowedge grouper growth is slower in the eastern GOM this

could result in greater impacts due to fishing since fish display a smaller size at age.

Reproductive and histological results supported previous literature that yellowedge grouper are monandric protogynous hermaphrodites (Keener, 1984; Bullock et al., 1996) similar to most grouper found in the GOM (Moe, 1969; Coleman et al., 1996; Collins et al., 1998). The presence of a single potential primary male, while notable, is not enough evidence to suggest that yellowedge grouper are diandric. The display of an asymmetrical size distribution between sexes and unequal sex ratios are additional characteristics of protogynous hermaphrodites (Moe, 1969; Coleman et al., 1996; Collins et al., 1998; Crabtree and Bullock, 1998; Wyanski et al., 2000).

Bullock et al. (1996) examined yellowedge grouper collected from 1977 to 1980 in the eastern GOM and Keener (1984) studied yellowedge grouper collected during 1977 to 1983 from South Carolina. In the GOM study females ranged in size from 360-1,065 mm TL (mean=676 mm TL) while males were larger, ranging from 580-1,083 mm TL (mean=880 mm TL) (Bullock et al., 1996). Samples from South Carolina ranged in size from 409-1,040 mm TL; females ranged from 409-990 mm TL and males ranged in size from 590 to 1,040 mm TL. Keener (1984) did not report mean sizes. Size ranges of yellowedge grouper from this study were similar; however, mean sizes were approximately 40 mm smaller for each sex illustrating the decrease in size over time. Bullock et al., (1996) reported that the capture of females >990 mm TL suggested that not all females transform into males, although current results question that idea. The lack of females collected in the older age classes (>33 years) suggested that either all females do transform into males or that females succumb to natural mortality.

Yellowedge grouper in the GOM appear to mature over a longer period of time

and at larger sizes than those in South Carolina. Keener (1984) reported immature females were 3-4 years old and ranged in size from 310-609 mm TL, although both age and sex information was obtained for only 19 females and 1 male. The smallest mature female identified was 409 mm TL while the smallest mature female found in the GOM was 475 mm TL. Keener (1984) reported mature females were 5-13 years old, whereas GOM yellowedge grouper were maturing between 2-16 years. She was able to provide both age and sex information for only one eleven year old male. Keener (1984) found that all yellowedge grouper ≥610 mm TL had reached sexual maturity, whereas GOM yellowedge grouper were all mature by ≥640 mm TL.

Yellowedge grouper in the GOM were sexually mature at smaller sizes than previously observed. Current size at 50% maturity (512 mm including all females and 543 mm for only active females) was considerably smaller than that reported by Bullock et al. (1996). Bullock et al. (1996) only used active females to estimate maturity and determined that 50% of the females in the GOM population reached sexual maturity at 569 mm TL. The decrease in size at maturity is consistent with the pervious size differences observed over time. Considerable size differences at maturity were observed when comparisons were made between the eastern and western GOM. Yellowedge grouper in the western GOM reached maturity at larger sizes than those in the eastern GOM which is consistent with earlier observed size differences. However, the lack of samples resulted in extremely large confidence internals questioning the significance of the observed difference. Additional sampling is suggested to determine if true differences exist.

Bullock et al. (1996) reported that transition from female into male is thought to

be a function of size. Transition is thought to occur rapidly due to the scarcity of transitional fish found. Bullock et al. (1996) reported that 50% of the females in their samples had transformed into males by a total length of 817 mm which is slightly smaller than the 840 mm currently observed but the size range is similar. However, when a direct comparison was made using only samples from the eastern GOM it appeared that the size at transition had decreased over time. Eastern GOM yellowedge grouper reached size at 50% transition at 811 mm TL. This direct comparison is more appropriate since Bullock et al. (1996) only collected fish from the eastern GOM. A decreased size at sexual transition is expected of an exploited stock (Coleman et al., 1996). Although results indicated that western GOM yellowedge grouper transitioned at larger sizes and older ages, the small sample size and large confidence intervals prevented conclusive results. Since differences in age and length have been observed between the eastern and western GOM a larger size at transition for western GOM fish might also be expected.

Another significant change over time is that of the sex ratio. Sex ratios for yellowedge grouper harvested 25 years ago in both the GOM and Atlantic indicated that this species had a larger proportion of males than currently observed. Bullock et al. (1996) reported the sex ratio of yellowedge grouper harvested from 1977-1980 was 1:1.8 (male:female). Similar results were reported by Keener (1984) who found the sex ratio for yellowedge grouper harvested from 1977-1980 to be approximately 1:2. Currently, 22% of yellowedge grouper from the eastern GOM were males as opposed to 36% males observed during 1977-1980 (Bullock et al., 1996). Recent data indicated a 14% decrease in the number of males in the population which can be an early sign of excess fishing pressure (Coleman et al., 1996). Sex ratios of other grouper in the GOM and Atlantic

vary greatly depending on species; however, a decline in the proportion of males appears to be a common occurrence. Coleman et al. (1996) reported that the current proportion of male gag, *Mycteroperca microlepis*, from the eastern GOM was 1%, decreasing from 17% in 1977. Black grouper sampled in South Florida from 1994-1996 had a male:female sex ratio of 1:15.4 (Crabtree and Bullock, 1998). Males represented only 1.2% to 22.9% of snowy grouper, *Epinephelus niveatus*, sampled from 1973-1994 in North Carolina and South Carolina (Wyanski et. al., 2000). Although the number of male yellowedge grouper in the population is considerably higher than that of many grouper species, the decline should not be overlooked. While significant differences were not observed between the sex ratio of eastern and western GOM yellowedge grouper, slightly more males were observed in the western GOM. Unfortunately, historical information on the sex ratio of western GOM yellowedge grouper was unavailable for direct comparison; however, it is possible the same trends are also occurring in the western GOM.

Variability of sex ratios between species may be due to reproductive behavior. Coleman et al. (1996) proposed that fish that form spawning aggregations are more susceptible to fishing pressure and consequently may have highly skewed sex ratios. Males tend to be removed first because they are larger and directly targeted. Males are also reported to be more aggressive towards bait than females making them more vulnerable to capture (Gilmore and Jones, 1992). Gag, which form spawning aggregations, have reported skewed sex ratios as low as 1:49 (male:female) (Collins et al.,1998). In contrast, Moe (1969) and Coleman et al. (1996) reported a male:female sex ratio of 1:6 and 1:2 to 1:4, respectively, for red grouper which do not form spawning aggregations. It is unknown whether yellowedge grouper form spawning aggregations,

however, males and females do appear to live in the same vicinity year round based on numerous captures of both sexes within a nautical mile. This could explain the higher number of males that appear to naturally occur in the population. The sex ratio should continue to be monitored to determine if it continues to skew and become more comparable to that of other exploited grouper in the GOM. The continued loss of males from the population could result in sperm limitation and reduced spawning success (Huntsman and Schaaf, 1994; Alonzo and Mangel, 2004). Since size limits or slot limits cannot be applied as management tools, due to the depth range of yellowedge grouper, other management strategies may need to be implemented if the number of males in the population continues to decline.

GOM yellowedge grouper displayed a protracted spawning season from March through September with peak spawning occurring July through September based on GSI values. This is slightly shorter than previously reported by Bullock et al. (1996) who reported the spawning season from January through October with peak spawning occurring May through September. Although gonadal development was observed in January, fish were not observed as fully mature until March. Keener (1984) reported a similar protracted spawning season from April through September for South Carolina yellowedge grouper. A longer spawning season occurring over the spring and summer months appeared similar to that of snowy grouper (Wyanski et al., 2000), and may also hold true for other less studied deep-water grouper species. This is in contrast to most shallow-water grouper such as red grouper and gag which spawn in the winter months (Moe, 1969; Collins et al., 1998).

Prior to this study there was no information available regarding yellowedge

grouper spawning frequency. The protracted spawning season of yellowedge grouper and the presence of several different oocyte stages in ovaries during the spawning season indicated that this species, like many other grouper species, is an indeterminate spawner (Hunter and Macewicz, 1985). Other examples of multiple-spawning grouper species in the southeastern U. S. Atlantic Ocean and Gulf of Mexico are the black grouper, gag, red grouper, goliath grouper and scamp (Moe, 1969; Bullock et al., 1992; Collins et al., 1998; Crabtree and Bullock, 1998; Harris et al., 2002). Spawning frequency varied depending on time of year and method of estimation. Sample size greatly influenced the results of the POF method which predicted considerably fewer spawning events than the FOM method. Estimates of spawning frequency have also varied for other GOM grouper. Gag were reported to spawn an average of 8 to 27 times per year, however, spawning frequency varied greatly on an annual basis and by fish age. Older fish spawned more frequently than younger fish. Consequently some age classes spawned over 40 times per year (Collins et al., 1998). Red grouper spawning frequency averaged 5 to 6 times per year but also varied by method with annual frequencies between 3 to 26 times per year (Collins et al., 2002; Fitzhugh et al., 2006). The loss of older, larger yellowedge grouper from the population could have significant impacts on spawning. If younger fish tend to spawn less frequently, the loss of older fish could result in a decrease in population size due to lower reproductive potential and eventually lead to overfishing.

The lack of gonads containing POF suggested that once yellowedge grouper spawn they either migrate to areas not fished or most likely do not actively feed for at least 24 hours making them not vulnerable to fishing. Histological evidence also supported more frequent spawning as oocytes undergoing FOM were observed in gonads

with POF. The POF were older than 24 hours indicating that yellowedge grouper do not spawn on a daily basis. Although spawning frequency estimates vary greatly, yellowedge grouper may spawn more often than many shallow-water grouper due to the lengthy spawning season. However, the occurrence of early maturing fish in June and July, and regenerating fish during most months, suggested that either all fish do not spawn annually or during the entire season as some appear to begin later while others end sooner.

Unfortunately, the loss of the gonads collected due to Hurricane Katrina prevented fecundity analysis in this study. In instances where fecundity data were absent or lacking, the use of gonad weight verses total weight, total length or age was used as a proxy for fecundity. The 2002 red grouper stock assessment (NMFS, 2002) used gonad weight verses age to calculate fecundity at age. The use of gonad weight provided for larger sample sizes since fecundity estimates were sparse (< 40 batch fecundity estimates per age group) (NMFS, 2002). Even if yellowedge grouper fecundity had been estimated, data were only available for thirty-four females ranging in age from 7-26 years old which excluded both the youngest and oldest age groups. The use of gonad weight nearly doubled the sample size and covered a wider age range. It is still desirable to estimate fecundity in the future, however, at present the use of gonad weight verses length, weight or age followed similar trends as that of batch fecundity estimates for other grouper. Gonad weight increased as age and length increased similar to the increase in fecundity as fish get older and larger (Hislop, 1988; Collins et al., 1998; Harris et al., 2002; Rhodes and Sadovy, 2002). Fecundity was shown to increase linearly as a function of body weight in camouflage grouper (Rhodes and Sadovy, 2002) while gonad weight increased linearly as whole weight increased in yellowedge grouper.

Results supported the importance that larger, older individuals have in the role of reproduction and perpetuation of this species.

Yellowedge grouper fecundity may be impacted in situations where all eggs were not resorbed and became a hardened mass in the gonad. Bullock and Smith (1991) noted a "hard, waxy material" in several yellowedge grouper gonads which was probably unshed eggs. Yellowedge grouper are not the only grouper observed to experience this condition; it has also been reported in scamp, goliath grouper, yellowmouth grouper, *Mycteroperca interstitialis*, and graysby, *Cephalopholis cruentata*, (Bullock and Murphy, 1994). This condition could impact annual fecundity since the mass can occupy a large region of the gonad, resulting in a reduced area for viable oocyte development.

It appeared that yellowedge grouper may be following the patterns of other exploited grouper found it the GOM. Significant changes in the age, length and sex structure have been observed, particularly in the eastern GOM, since the onset of fishing. Although little change in length has occurred since 1991 suggesting length changes to the population structure as a result of fishing may have reached a plateau, the number of older individuals observed has continued to plummet. Considerable differences were observed between the current population structure of yellowedge grouper from the eastern and western GOM. This finding may require different management practices for each of the regions.

Grouper are thought to be sensitive to exploitation since many are slow growing, long-lived and hermaphrodites (Coleman et al., 1999; Musick, 1999; Coleman et al., 2000), and yellowedge grouper appear to be no exception. Yellowedge grouper may be unusually sensitive to fishing since they have such a long lifespan and slow growth rate.

Species with *K* coefficients <0.10 appear to be particularly vulnerable to fishing pressure (Musick, 1999). The early warning signs of overfishing (reduction in mean size and age, loss of older individuals and change in sex ratio) have already begun. Closed fishing seasons or reduced annual catch quotas are management actions that could be implemented to improve population status. Continued monitoring of life history parameters is necessary to ensure continued survival of this species in the GOM.

CHAPTER III

DISTRIBUTION, STOCK DESCRIPTION AND ABUNDANCE

Introduction

Yellowedge grouper, *Epinephelus flavolimbatus*, are reported to inhabit a variety of habitats in offshore waters (90-365 m) throughout the Gulf of Mexico (GOM) (Smith, 1971). Currently, there are limited published scientific studies describing yellowedge grouper distribution, environmental preference, stock description and abundance in the GOM. To adequately assess a species, fishery independent surveys with random station selection over the known range of the population are usually necessary.

Since 1967, the NMFS (National Marine Fisheries Service), Mississippi Laboratory (MS Lab) has conducted a variety of surveys in United States, Caribbean, Central American and South American waters using numerous gear types. Beginning in 1973, semi-annual Southeast Area Monitoring and Assessment Program (SEAMAP) trawl surveys were conducted during June-July and October-November between 9-110 m from 88º W to 97.5º W. During 1968-1987, several MS Lab fishery independent surveys were conducted to evaluate the deep-water snapper, grouper and tilefish stocks. Bottom longlines and off-bottom longlines, which fished approximately 2-8 m from the bottom, fish traps and trawls, were used. Beginning on the 1979 longline cruise individual fish data (total length and weight) were recorded and otoliths were occasionally collected and archived.

Recently, fishery independent data were collected during GOM bottom longline surveys initially designed to assess distribution and relative abundance of coastal sharks in the western North Atlantic Ocean and GOM. Beginning in 1999, survey

objectives were expanded to include red snapper (*Lutjanus campechanus*) and other important commercial and recreational fish. Survey depths were expanded to sample from 9-183 m. In 2001, the survey was modified to sample from 9-366 m in order to sample deep-water grouper and tilefish species. Fishing effort for the 2001-2004 surveys was proportionally allocated by depth strata with 50% of the effort in 9-55 m, 40% in 55-183 m and 10% in 183-366 m. Gear consisted of an approximately one nautical mile bottom longline with 100 #15/0 circle hooks baited with Atlantic mackerel, *Scomber scombrus*, which was soaked for one hour (Jones, 2001).

Data collected during these studies were used to describe the distribution, habitat preferences and relative abundance of yellowedge grouper in the northern GOM. Results were used to examine the null hypothesis that yellowedge grouper are evenly distributed throughout the GOM. In addition, the relationship between size and depth was analyzed to test the null hypothesis that yellowedge grouper are randomly distributed according to depth.

Since yellowedge grouper are reported to inhabit the entire GOM it is important to distinguish if the current population is composed of a single stock or by multiple stocks throughout the GOM. Cass-Calay and Bahnick (2002) assumed that the northern GOM (United States waters), southern GOM (Mexican waters) and Atlantic Ocean were all separate stocks; however, they did not investigate if there was a stock difference between yellowedge grouper within the northern GOM. A stock can be defined as species with a common gene pool that inhabits a specific geographic area and has unique growth and mortality parameters (Sparre and Venema, 1998). Management of separate stocks of lutjanid fishes in the southern GOM was suggested by Arreguin-Sanchez and

Manickchand-Heileman (1998). The final objective of this study was to evaluate the null hypothesis that only one stock of yellowedge grouper occurs in the northern GOM (from hereon referred to as GOM). Age, weight and length structure, density and abundance were compared between two regions and three zones in order determine if there was more than one stock. If multiple distinct stocks exist in the GOM it may be necessary to employ different regulations for each stock.

Methodology

The GOM was divided into three Zones, eastern (E), central (C) and western (W) (Figure 32), based on NMFS Faunal Zones (NMFS, 2001). The GOM was also divided into two regions, eastern GOM and western GOM, at the 89° 15' W longitude for comparisons between regions. The eastern GOM region was comprised of Zones E and C and the western GOM region was Zone W. Yellowedge grouper collected were assigned to each Zone and region based on location of capture.

Data from historical and current MS Lab cruises were plotted to determine the distribution of yellowedge grouper in the GOM. Capture locations were plotted on bathymetric maps to determine habitat preference. A two-sample *t*-test was used to determine if there was significant difference between the mean total length of yellowedge grouper captured in trawls (shrimp, fish, mongoose and high opening bottom trawls were all combined) verses longlines (bottom, off-bottom and vertical drift longlines were combined). The correlation between total length verses depth was investigated to establish if there was a specific size structure associated with depth. In cases where a start and end depth was recorded, the mean depth was used. Only yellowedge grouper collected from 1982-2004 were measured and used for the analyses.

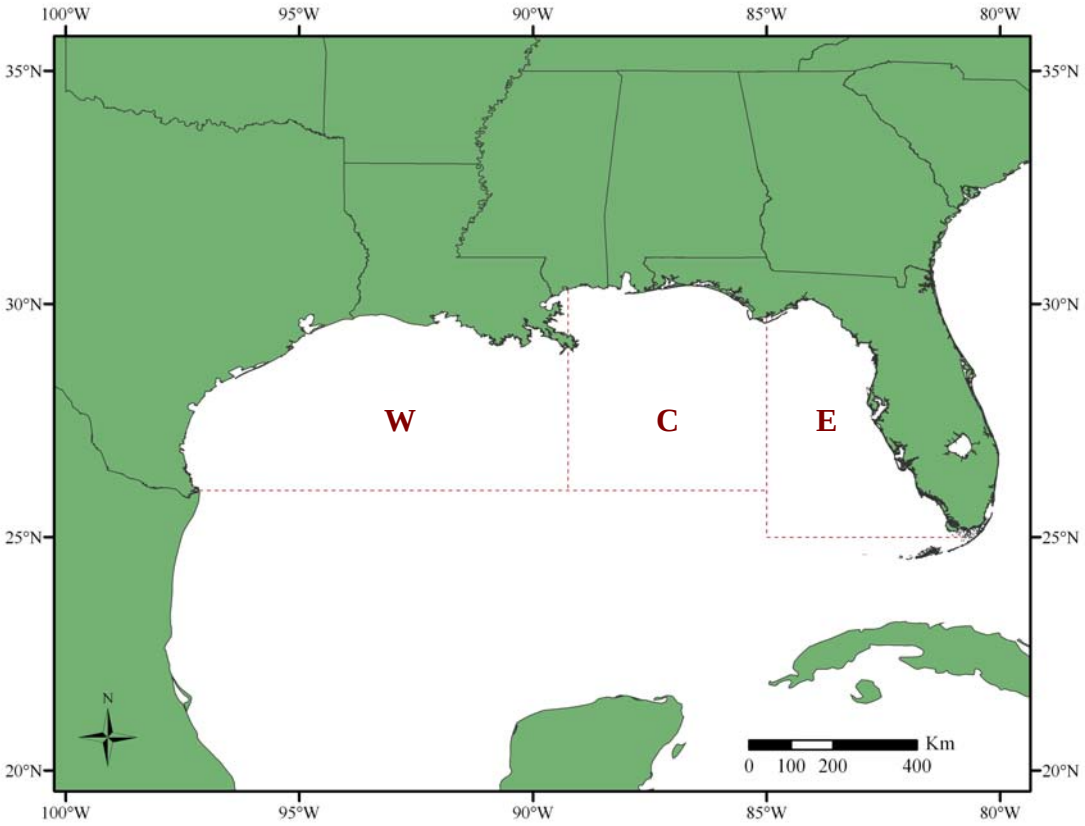

Figure 32. National Marine Fisheries Service Faunal Zones. W refers to the western Gulf of Mexico, C to the central Gulf of Mexico and E to the eastern Gulf of Mexico.

Numerous survey designs were used during historical studies, and some surveys were directed without random station selection. Therefore, only data from the 1999-2004 bottom longline surveys were used for the following analyses. A chi-squared test with Sequential Bonferroni comparisons was used to determine if the proportion of captures (i.e. successful/unsuccessful) varied between Zones. A Kolmogorov-Smirnov (K-S) two-sample test was used to determine if there was a significant difference between the length frequency distributions of yellowedge grouper collected in the eastern and western GOM regions. Independent samples *t*-tests were used to determine if mean total length, weight

and age of fish captured were significantly different between the eastern and western GOM regions. A one-way ANOVA was used to determine if the mean total length, weight and age of fish differed significantly among Zones.

Catch-per-unit-effort (CPUE) from the 1999-2004 bottom longline surveys was used to calculate an index of relative stock abundance. CPUE of yellowedge grouper, expressed as the number of fish caught per 100 hooks per hour, was calculated annually for the northern GOM, each region and Zone. Only stations that occurred in greater than 50 m depths were used for the analysis. Comparisons of CPUE from the eastern and western GOM regions and between Zones were made using a *t*-test and one-way ANOVA, respectively, to determine if there was a difference in abundance between areas.

Statistical analysis was conducted using SPSS 14.0 (SPSS, Inc., Chicago, IL) or SigmaStat 3.5 (Systat Software Inc., Point Richmond, CA). All data were tested for normality and homogeneity of variance. If data were not normally distributed a natural log transformation was used to normalize the data. Nonparametric tests were conducted if there were large violations of these assumptions. Results were considered significant if $P<0.05$.

Results

A total of 1,156 yellowedge grouper were captured at 529 stations from 1967-2004. The majority of captures occurred in the GOM, remaining captures occurred outside United States waters, where 905 yellowedge grouper were reported at 434 stations. It was possible that the number of yellowedge grouper collected using shrimp trawls was inflated due to data extrapolation of sub-sampled catch since several stations

reported 4-7 individuals per station; a rare occurrence in trawls (personal observation). Shrimp trawls (43.8%) and bottom longline gear (40.3%) collected the majority of specimens.

Yellowedge grouper were primarily distributed between the 50 to 300 m depth contours (mean=124.7 m, SE=3.46; Figure 33) throughout the GOM.

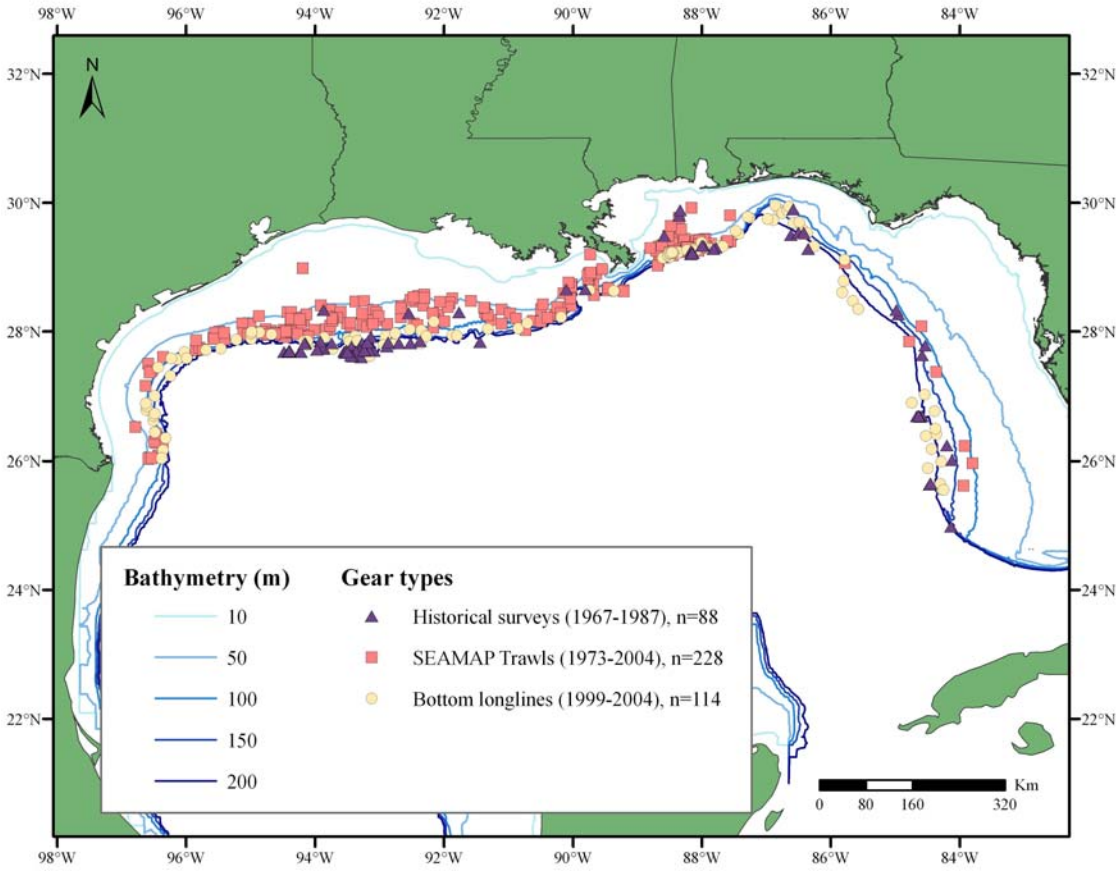

Figure 33. Locations of yellowedge grouper collected on fishery independent surveys from 1967-2004. Gear types used include trawls (shrimp, fish, high opening bottom and mongoose), longlines (vertical, off-bottom and bottom) and fish traps. Data points indicate location of catch not number of fish collected.

SEAMAP trawl surveys mainly conducted from Texas to the Alabama-Florida boarder collected yellowedge grouper in 11-283 m (mean=81.3 m, SE=2.48). Recent bottom longline surveys and historical MS Lab surveys (which primarily used longline gear) captured yellowedge grouper in 73-296 m (mean=145.7, SE=4.70) and 36-391 m (mean=208.1, SE=8.27), respectively. Yellowedge grouper capture locations were overlaid on topographic maps provided by Doug Weaver, NOAA Fisheries Service, to discern preferred habitat (Figure 34A-E). Stations were conducted on both hard and soft substrate; however, yellowedge grouper appeared to prefer mostly soft substrate throughout the western and central GOM. Habitat maps were not available for the entire eastern region.

Bottom temperature of capture locations ranged from 10.7°C to 27.0°C (mean=18.5 °C, SE=0.22) but varied considerably by survey. Yellowedge grouper collected on SEAMAP trawl surveys were collected in shallower, warmer bottom temperatures (mean=21.0 °C, SE=0.26, range=13.5-27.0) than those on both the historical and current longline surveys (mean=15.4 °C, SE=0.74, range=11.2-21.3 and mean=17.0 °C, SE=0.26, range=10.7-22.7, respectively). Bottom salinity also varied by survey with the greatest range (25.3‰ to 38.0‰) occurring on SEAMAP trawl surveys. However, the average salinity for both the historical and SEAMAP surveys was 36.1‰ and 36.2‰ on the current bottom longline surveys indicating comparable salinity preferences. Yellowedge grouper are tolerant of lower dissolved oxygen conditions and were collected in waters ranging from 2.1 to 9.6 mg/L (mean=4.6 mg/L, SE=0.07).

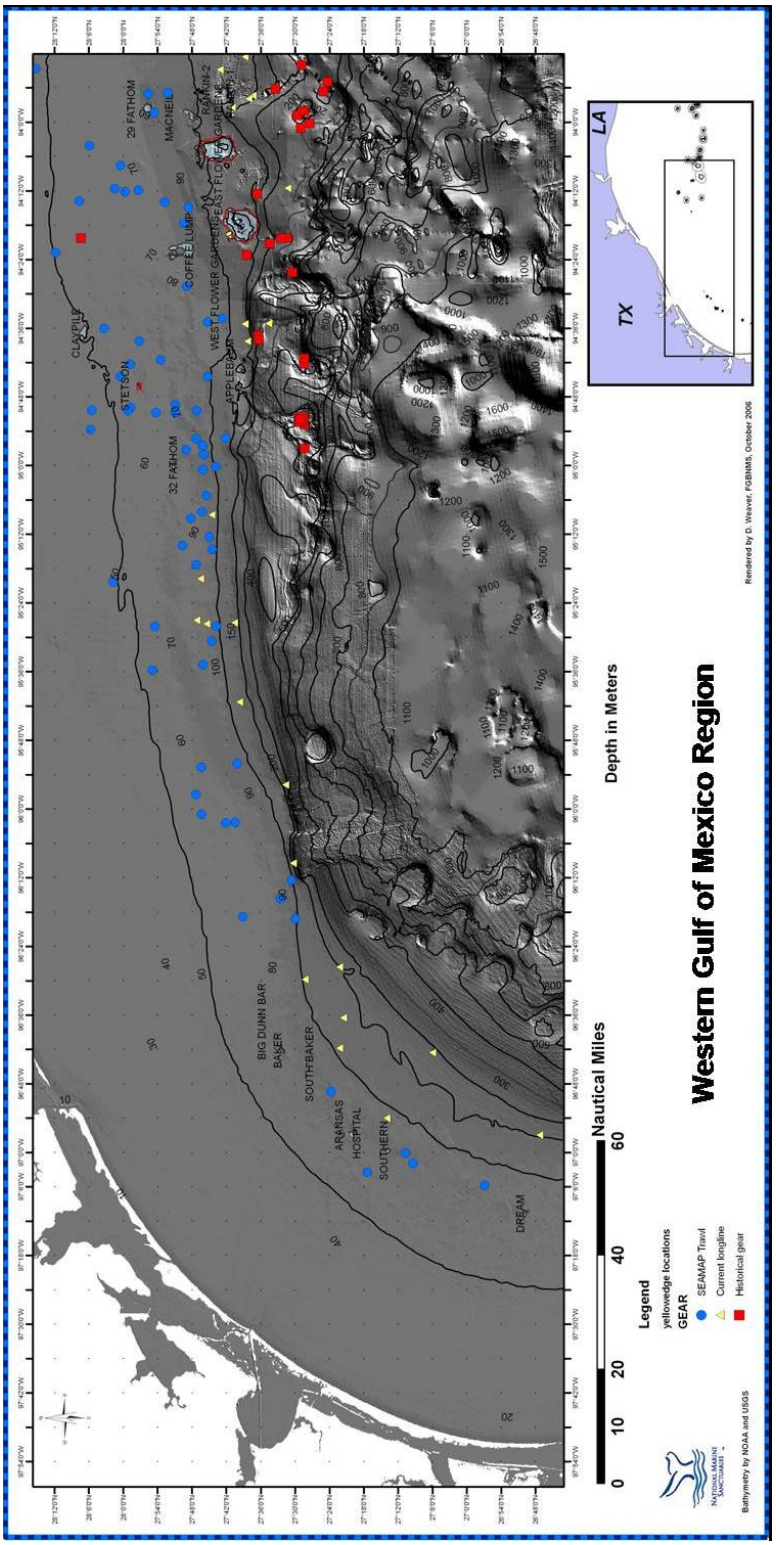

Figure 34 A. Yellowedge grouper collected in the western Gulf of Mexico on fishery independent surveys from 1967-2004. Gear used included trawls, bottom longlines and historical gear which were primarily longlines. Data points indicate location of catch not number of fish collected.

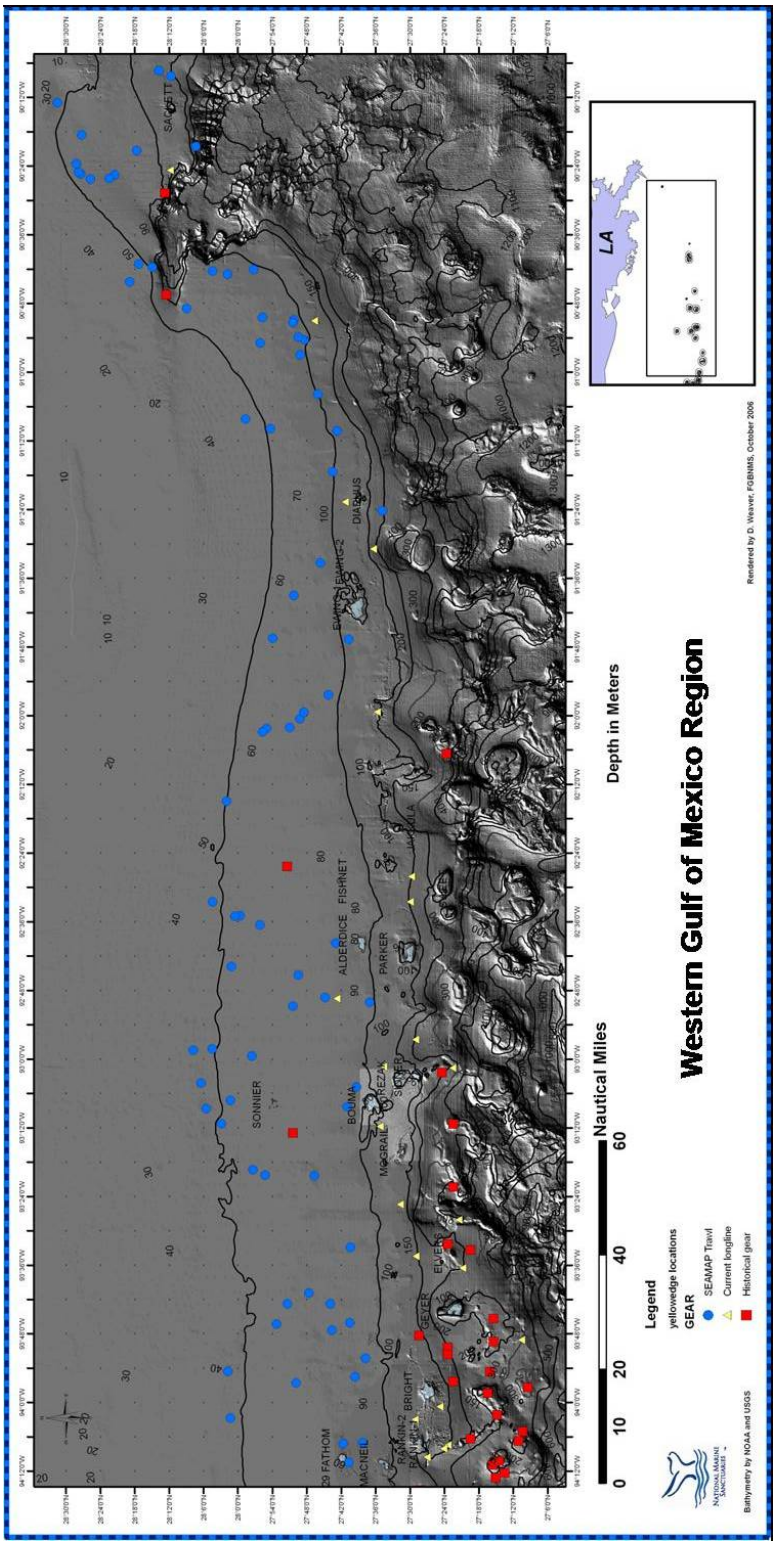

Figure 34 B. Topography of yellowedge grouper capture locations. Yellowedge grouper collected in the western Gulf of Mexico on fishery independent surveys from 1967-2004. Gear used included trawls, bottom longlines and historical gear which were primarily longlines. Data points indicate location of catch not number of fish collected.

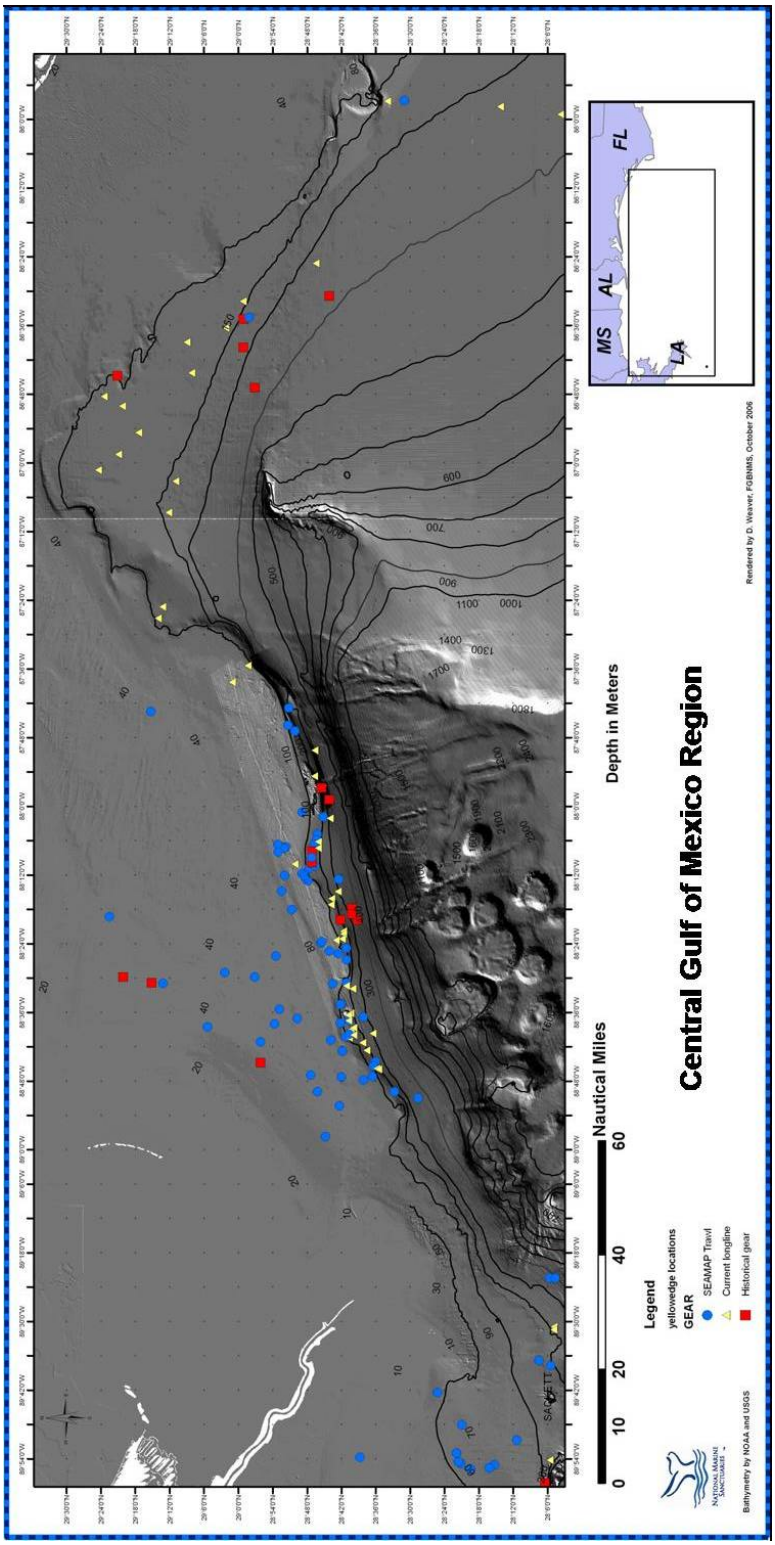

Figure 34 C. Topography of yellowedge grouper capture locations. Yellowedge grouper collected in the central Gulf of Mexico on fishery independent surveys from 1967-2004. Gear used included trawls, bottom longlines and historical gear which were primarily longlines. Data points indicate location of catch not number of fish collected.

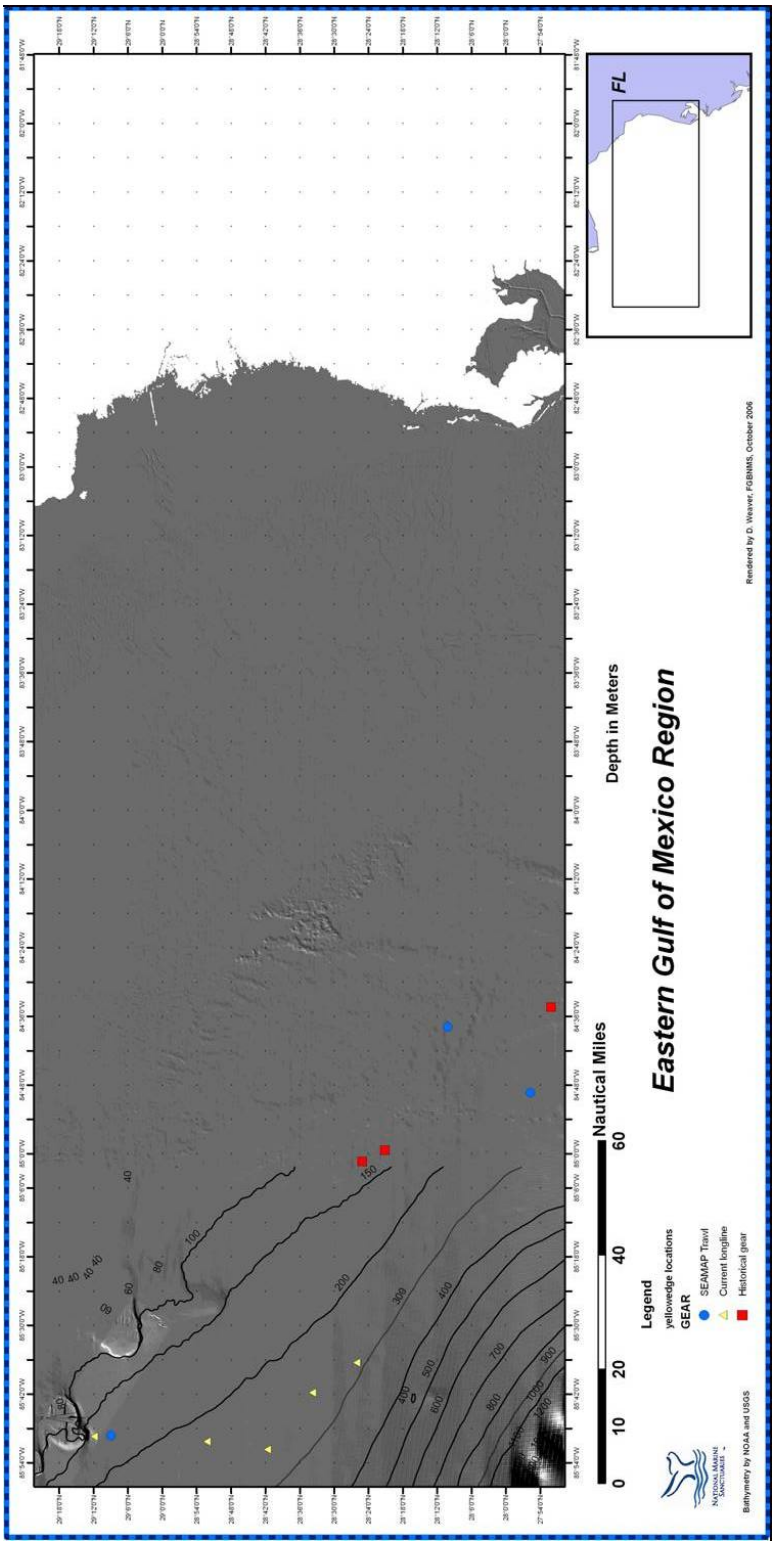

Figure 34 D. Topography of yellowedge grouper capture locations. Yellowedge grouper collected in the eastern Gulf of Mexico on fishery independent surveys from 1967-2004. Gear used included trawls, bottom longlines and historical gear which were primarily longlines. Data points indicate location of catch not number of fish collected.

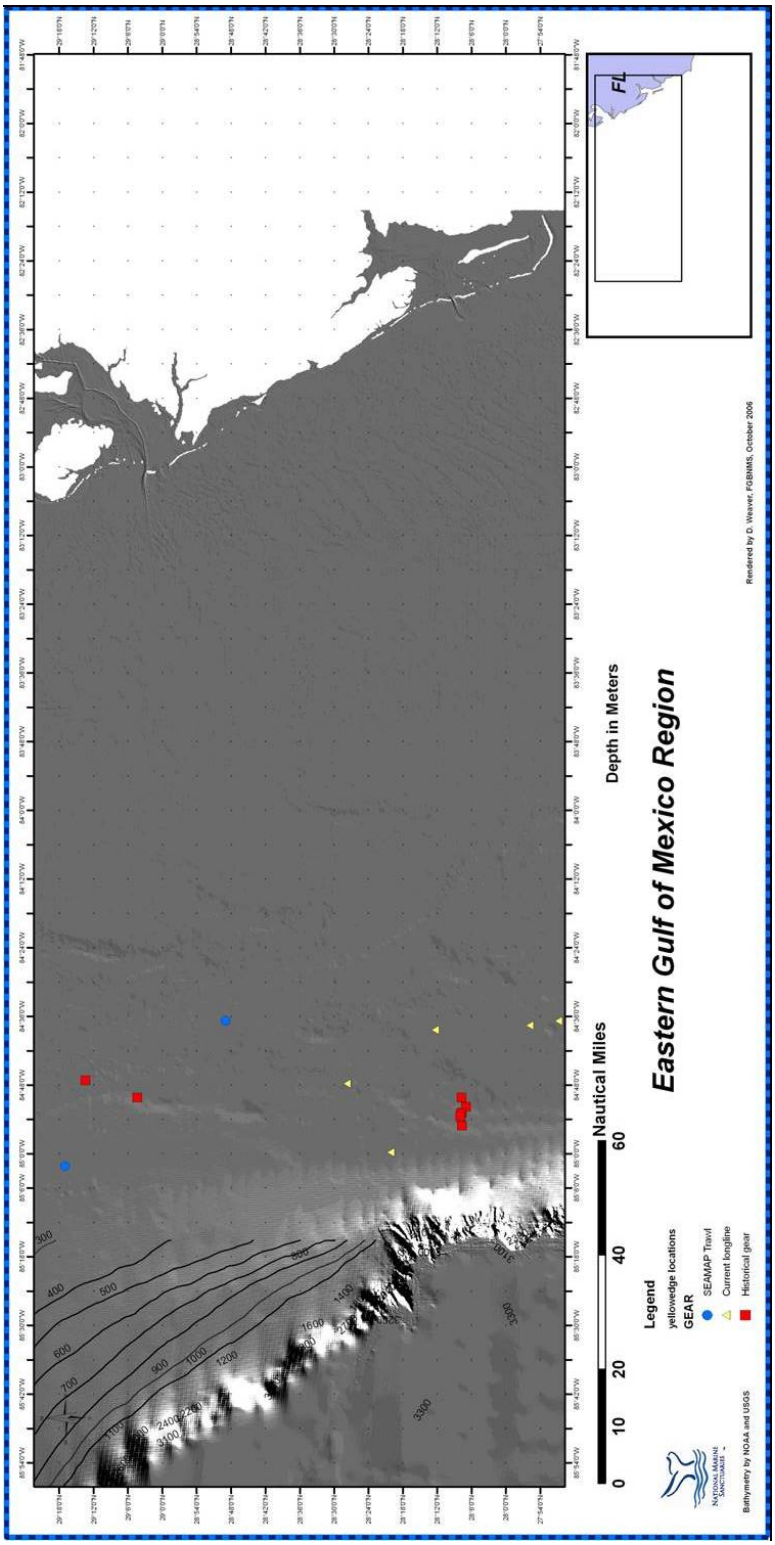

Figure 34 E. Topography of yellowedge grouper capture locations. Yellowedge grouper collected in the eastern Gulf of Mexico on fishery independent surveys from 1967-2004. Gear used included trawls, bottom longlines and historical gear which were primarily longlines. Data points indicate location of catch not number of fish collected.

Length data were only available for yellowedge grouper collected in 11 to 300 m depths. A significant difference (*t*-test, t=25.19, P<0.001) in total length was detected between fish captured using shrimp trawls (n=100, mean=218.3 mm) verses longline gear (n=312, mean=705.3 mm; Figure 35).

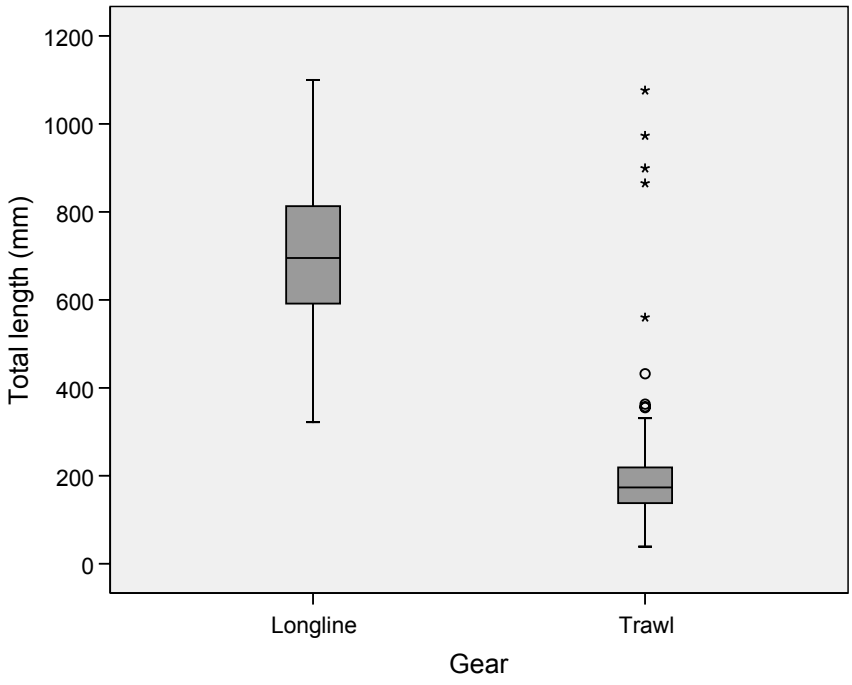

Figure 35. Median total length by longline (n=312) and trawl (n=100) gear of yellowedge grouper collected from 1982-2004 in the northern Gulf of Mexico. The box length represents the interquartile range, the open circles are outliers and the * represents extreme cases.

The relationship between total length (TL) and depth was best expressed as a power function (Depth=5.315*$TL^{0.515}$; Figure 36). Smaller yellowedge grouper (<400 mm TL) were found in shallower depths between 35-125 m while larger fish were found in up to 300 m depths. A large amount of variability (R^2=0.467) between TL and depth

was observed, indicating that once fish reached >400 mm TL they were found at any depth between 125-300 m.

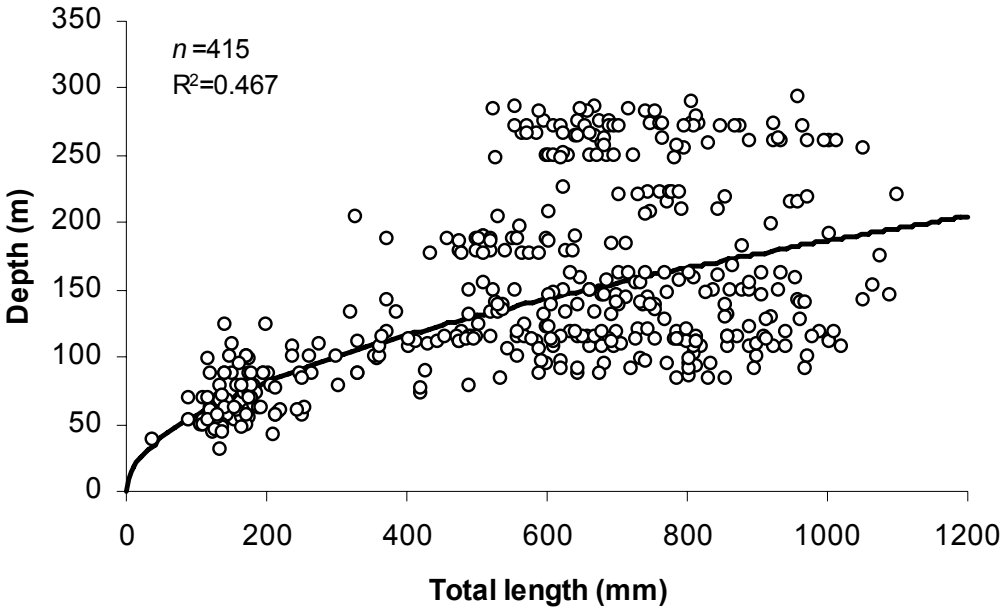

Figure 36. Yellowedge grouper depth verses total length relationship. Relationship expressed as Depth=5.315*$TL^{0.515}$. Yellowedge grouper were collected from 1982-2004 using longline and trawling gear on fishery independent surveys in the northern Gulf of Mexico.

The 2000-2004 bottom longline surveys were designed to annually sample the entire GOM; only the 1999 survey was designed with limited coverage in the central GOM. However, survey effort and catch varied annually by area (Table 16); some Zones experienced little or no effort due to weather, mechanical problems or time constraints. A total of 723 stations were completed resulting in 235 yellowedge grouper captures at 110 stations (Figure 37). The majority of effort occurred in Zone W (43.0%) followed by Zone C (37.2%) and Zone E (19.8%). Zones W and C had significantly (X^2=6.444,

P=0.04; Bonferroni, *P*>0.05) higher percentages of positive captures (17.0% and 16.7%, respectively) than Zone E (8.4%) (Bonferroni, *P*<0.05). However, once encountered in Zone E twice as many yellowedge grouper were collected per station (3.4 verses 1.7 per 100 hook hours) than in Zone W and nearly 1.5 times as many as in Zone C (2.3 per 100 hook hours; Figure 38). Density was only significantly different, however, between Zones E and W (ANOVA, $F_{2,107}$=5.59, *P*=0.005; Bonferroni, *P*=0.004).

Table 16. Summary of effort and yellowedge grouper collected by Zone, 1999-2004. Total number of stations completed (SS), number of stations that captured yellowedge grouper (PC) and number of yellowedge grouper collected (*n*), in the Gulf of Mexico (GOM) on fishery independent bottom longline surveys.

Survey Year	Total stations sampled (SS), Positive capture stations (PC), Yellowedge grouper collected (*n*)								
	Eastern GOM			Central GOM			Western GOM		
	SS	PC	(*n*)	SS	PC	(*n*)	SS	PC	(*n*)
1999				64	12	44			
2000	1	0	0	70	7	14	63	13	20
2001	31	0	0	35	5	7	74	12	21
2002				35	10	15	83	13	24
2003	56	7	27	38	7	15	48	8	11
2004	55	5	14	27	4	8	43	7	15
Total	143	12	41	269	45	103	311	53	91
Percent effort/Zone	19.78			37.21			43.02		
Percent positive captures	8.39			16.73			17.04		
Mean *n*/positive capture	3.42			2.29			1.72		

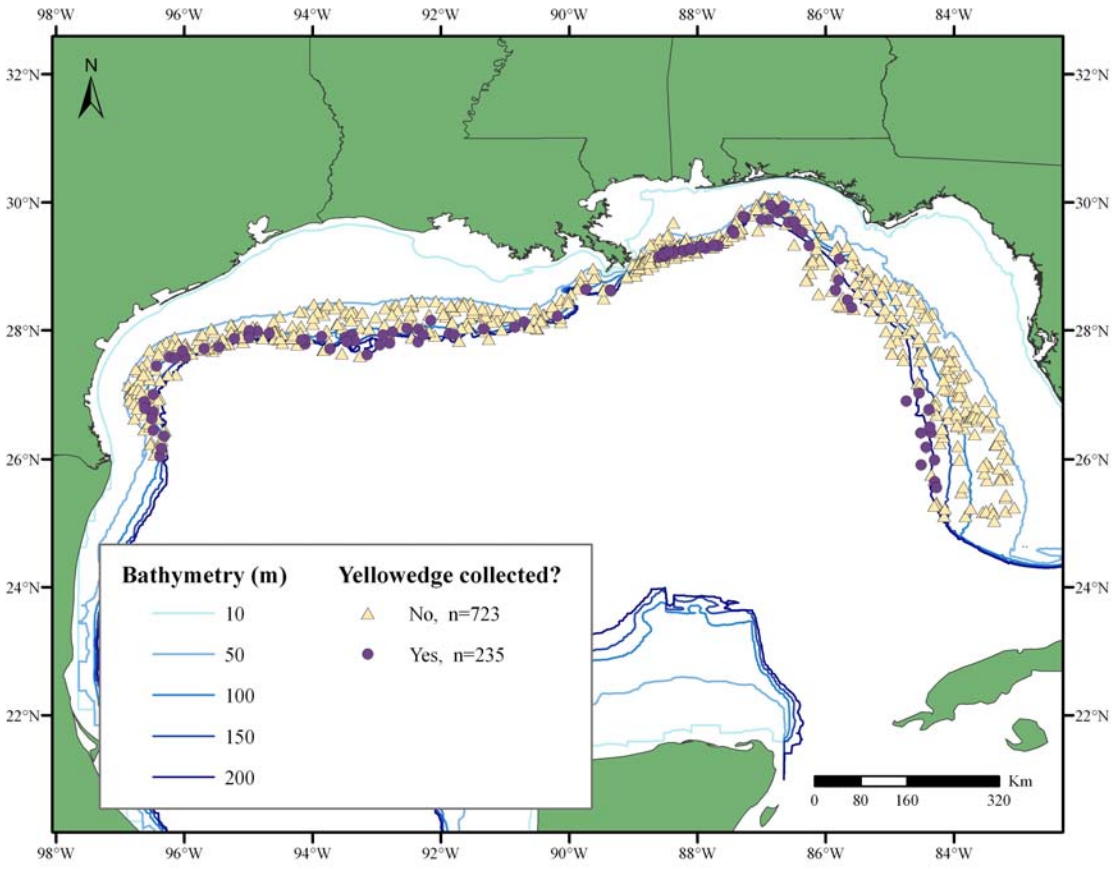

Figure 37. Locations of fishery independent bottom longline stations from 1999-2004. Stations where at least one yellowedge grouper was collected are represented with a circle.

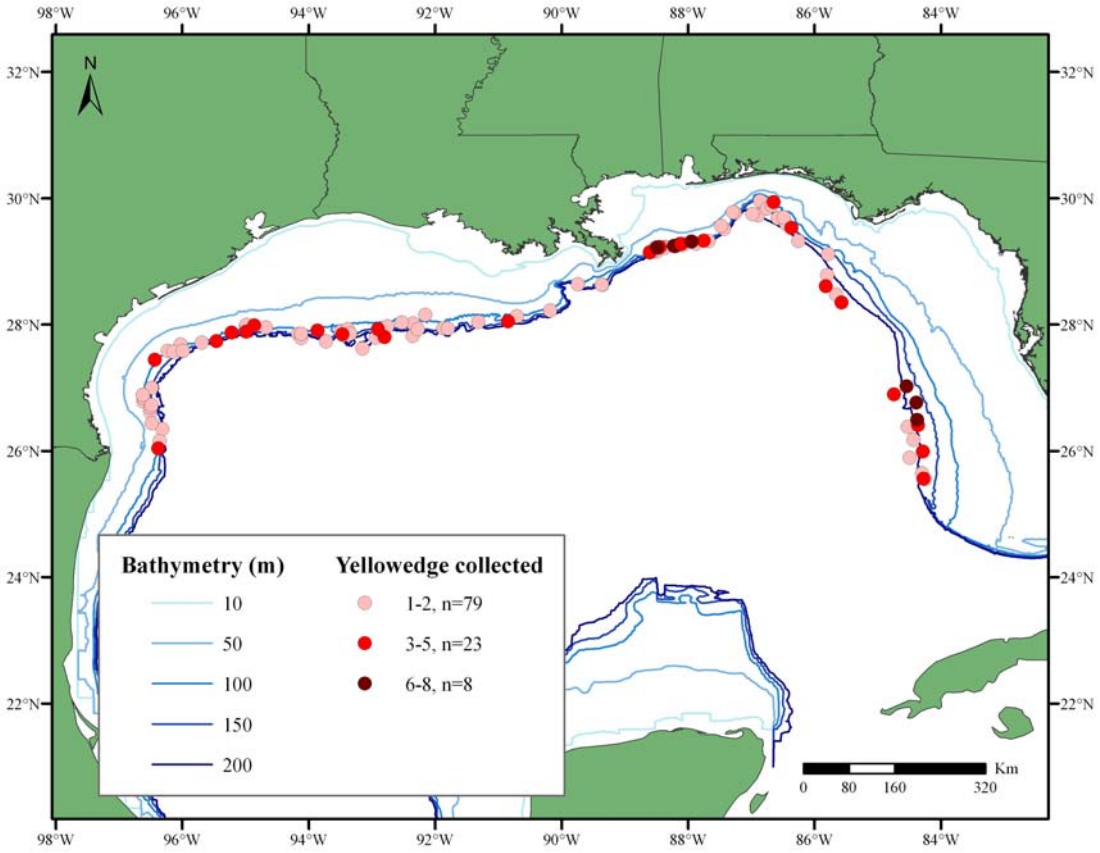

Figure 38. Density (number per 100 hooks per hour) of yellowedge grouper in the Gulf of Mexico. Yellowedge grouper were collected on fishery independent bottom longline surveys from 1999-2004.

The following comparisons were conducted between regions (Zones E and C were combined to form the eastern GOM region) as opposed to Zones. The length-frequency distributions of yellowedge grouper collected in the eastern and western GOM regions were significantly different (Two-Sample K-S test, n=223, D=2.901, P<0.001; Figure 39).

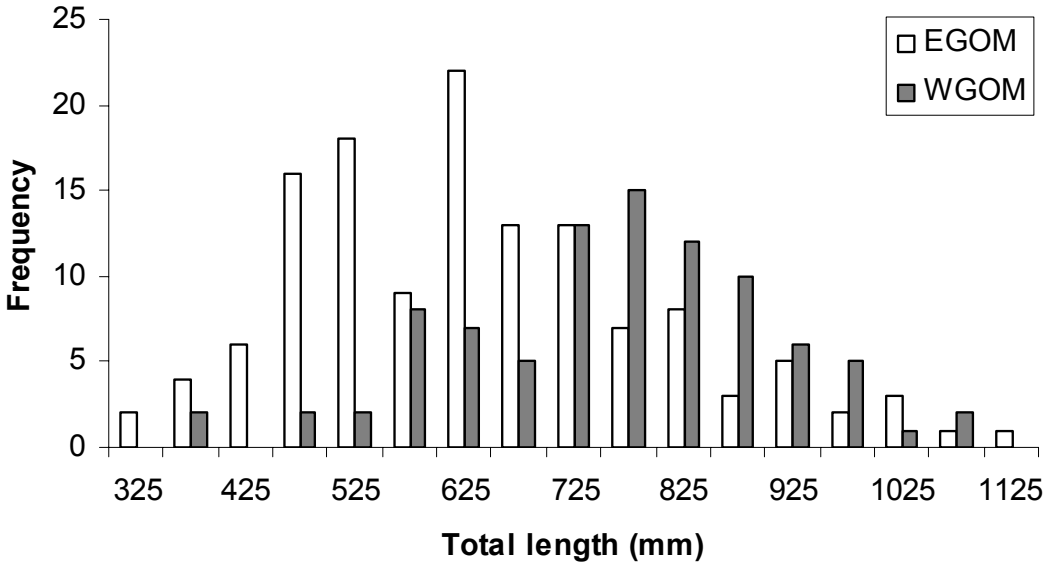

Figure 39. Regional length-frequency distributions of yellowedge grouper collected during 1999-2004 using fishery independent bottom longline gear. Regions included the eastern Gulf of Mexico (EGOM, n=133) and western Gulf of Mexico (WGOM, n=90). Total length indicates the midpoint of the size class.

Yellowedge grouper collected in the western GOM ranged from 373-1,065 mm TL (mean=759.1 mm, SE=14.92) and were significantly larger (t-test, t=-5.60, P<0.001) than those from the eastern GOM (mean=640.1 mm, SE=14.28, range 322-1,100). Fish in the western GOM (mean=3.7 kg, SE=0.26, range 0.4-16.0) were also significantly heavier (t-

test, t=-6.44, P<0.001) than those in the eastern GOM (mean=5.7 kg, SE=0.30, range 0.7-14.0). Mean ages of western GOM yellowedge grouper (18.3 years, SE=1.07, range 3-70) were significantly older (t-test, t=-5.42, P<0.001) than eastern GOM fish (12.1 years, SE=0.63, range 2-43).

Faunal Zone comparisons were used to evaluate if two or three separate stocks were present in the GOM. Zone W, which coincided with the western GOM, had the largest and oldest fish (ANOVA, $F_{2,220}$=17.89, P<0.001; $F_{2,214}$=17.56, P<0.001, respectively). There was no significant difference between lengths of fish captured in Zones E and C (Games-Howell, P=0.073), however, the mean age of fish from Zone E (mean=13.2 years, SE=0.92, range=7-31) was significantly older (Games-Howell, P=0.028) than yellowedge grouper from Zone C (mean=11.6 years, SE=0.81, range=2-43; Figure 40).

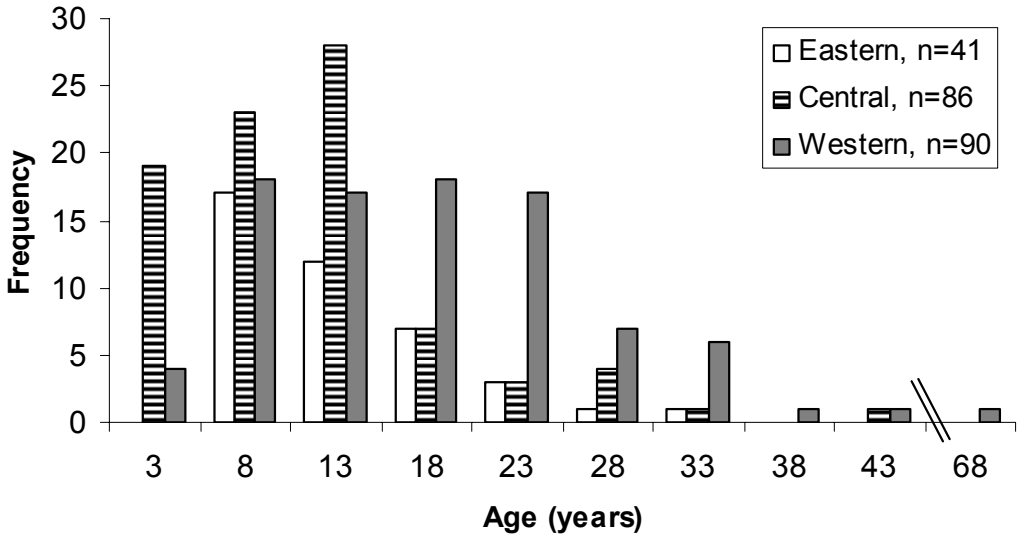

Figure 40. Age-frequency distributions of yellowedge grouper collected during 1999-2004 fishery independent bottom longline surveys in the Eastern, Central and Western Gulf of Mexico. Data labels indicate the midpoint of the size classes, 5 years per class.

Total weight data violated the homogeneity of variance assumption (Levene's Test, F=8.10, P=0.001), therefore, a Kruskal-Wallis test with Dunn's multiple comparisons was used to compare Zones. Significant differences were found between all three Zones ($H_{2,222}$=41.62, P<0.001), yellowedge grouper were heaviest in Zone W (median=5.3 kg) followed by C (median=3.4 kg) and E (median=2.3 kg).

CPUE of yellowedge grouper was calculated annually for the GOM and for each region (1999 was excluded from comparisons since it only contained stations in the central GOM). Data from Zone E was only available for 2003 and 2004; therefore, comparisons between Zones were not conducted. Relative CPUE (mean year CPUE/series mean CPUE) was calculated for comparisons between years since effort varied annually (Figure 41).

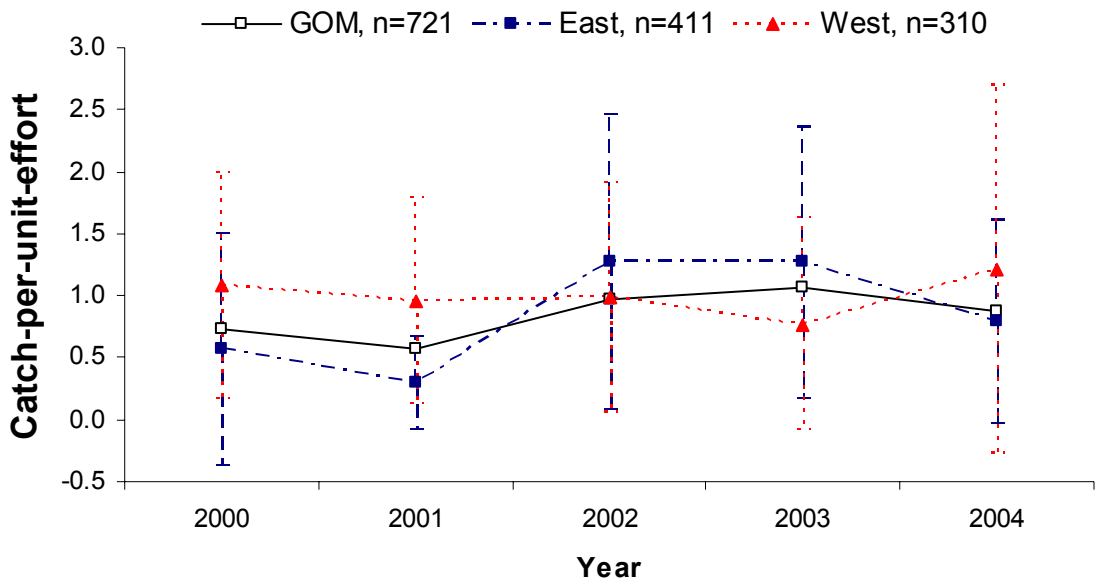

Figure 41. Annual relative yellowedge grouper catch-per-unit-effort (CPUE). CPUE is presented for the entire Gulf of Mexico (GOM), and eastern (East) and western (West) GOM regions. Error bars represent one standard error.

Relative CPUE in the GOM was reasonably constant from 2000-2004 and ranged from 0.7 to 1.1 yellowedge grouper per 100 hooks per hour. CPUE was low in 2000 and 2001, increased in 2002, peaked in 2003 and dropped slightly in 2004. CPUE varied annually by region. Catches were higher in the western GOM initially, followed by increased CPUE in the eastern GOM in 2002 and 2003, however, CPUE in the eastern GOM declined in 2004. CPUE was not normally distributed and numerous transformations did not normalize the data. Therefore, comparisons of CPUE from the eastern GOM and western GOM were made using a Mann-Whitney *U* test which determined there was no significant difference (z=-1.005, P=0.315) in median CPUE between regions.

Discussion

The adaptability of yellowedge grouper to various habitat types has resulted in a Gulf wide distribution unlike other grouper species which are found in greater abundance in the eastern GOM (Moe, 1963; Hose and Moore, 1992; Koenig et al., 2000; Franks, 2005) Yellowedge grouper are reported to occur in a variety of habitats possibly giving them an advantage over other grouper which are mainly associated with various hard substrates such as coral reefs, rocks, outcroppings, artificial reefs and ship wrecks (Jones et al., 1989; Heemstra and Randall, 1993, Huntsman et al., 1999). The GOM is composed of two principal sediment provinces, terrigenous or rock-derived sediment from Texas to the western part of the Desoto Canyon and carbonate sediment along the west Florida shelf (Pequegnat et al., 1990). Yellowedge grouper appear to have adapted to each region making them the dominant deep-water grouper in the GOM (Chester et al., 1984; Cass-Calay and Bahnick, 2002).

Off Texas yellowedge grouper are found over several different habitat types such

as flat bottom, near "lumps" associated with tilefish, *Lopholatilus chamaeleonticeps*, and in the proximity of topographic highs (Jones et al., 1989). Yellowedge grouper were also observed near rock outcroppings, rock ridge habitats and co-occurring with snowy grouper, *Epinephelus niveatus*, in areas with high densities of rocks (Roe, 1976; Jones et al., 1989). Western GOM yellowedge grouper were observed in three distinct types of burrows, similar to those associated with tilefish, cut into cohesive mud-clay sediment at depths of 265 to 290 m (Jones et al., 1989). They have also been found at the shelf edge on mud, sand or sand-shell bottom (Jones et al., 1989; Heemstra and Randall, 1993). In the central GOM yellowedge grouper appear to be associated with soft substrate near the Mississippi-Alabama pinnacles and also with patch reef areas within the pinnacles. Scalon et al. (2005) observed numerous pockmarks with burrows cut into sandy silty clay sediment located in the Madison-Swanson and north of the Steamboat Lumps Fishery Reserves along the Florida west coast. Although no yellowedge grouper were observed on ROV dives (Scanlon et al., 2005), the burrows resembled those seen in the western GOM (Jones et al., 1989) and were possibly constructed by yellowedge grouper. An ROV filmed yellowedge grouper along the top edge of the Naples sinkhole which is located approximately 240 km west of Naples, FL in 175 m of water (Reed et al., 2005). The edge was composed of hard bottom, small cobble (<0.1 m) and rock outcrops less than one meter high, and the surrounding substrate was flat with silty sand and 0.3-0.6 m rock talus (Reed et al., 2005). The highest densities of yellowedge grouper collected on MS Lab surveys were within a 45 km radius of the Naples sinkhole along the 100 Fathom Break suggesting there is preferred habitat in this area. In the Atlantic, yellowedge grouper appear to prefer hard substrate and were reported on shelf-edge habitat composed

of rocky ledges with vertical relief, jagged peaks and steep cliffs (Grimes et al., 1982).

In addition to adapting to various environments yellowedge grouper are considered to be "ecosystem engineers" (Jones et al., 1994) due to their construction of burrows which modify habitat and enhance the biodiversity and community structure of the outer continental shelf (Coleman and Williams, 2002). Yellowedge grouper and tilefish burrows are usually observed containing bioerosion as well as smaller burrows constructed by additional inhabitants such as galatheid crabs, cleaner shrimp, other invertebrates and small fishes (Able et al., 1982; Jones et al., 1989). The burrows create micro-habitats in outer shelf areas that are often void of life. The role of ecosystem engineers has recently been recognized but is not fully understood (Coleman and Williams, 2002). These sensitive ecosystems are vulnerable to human activities and could be severely impacted by oil and gas exploration in the deep ocean (Coleman and Williams, 2002).

It was not unexpected that trawls captured mostly small yellowedge grouper since smaller fish tend be less mobile and not as likely to avoid trawls as larger fish (Winger et al., 1999; Krause et al., 2005). Gear selectivity can also influence the size of fish captured. Trawls typically capture smaller fish than longline gear (Roe, 1976; Lokkeborg and Bjordal, 1992; Clarke et al., 2002). Most species of grouper experience ontogenetic depth preferences where juveniles occupy near-shore or estuarine habitats and migrate to deeper water as adults (Moe, 1969; Coleman et al., 1999, Renan et al., 2003). It appears that juvenile yellowedge grouper begin their lives in shallower water and migrate to deeper depths by the time they reach 300 to 450 mm TL. Although adult yellowedge grouper were found in a variety of depths most fish were found between 100 to 200 m

isobathys in the northern GOM.

CPUE remained fairly constant from 1999 to 2004; fluctuating the most in the eastern GOM. Results suggested that no major trend in fishery independent landings occurred from 2000 to 2004 and that relative abundance was similar in both regions. However, the data contained a moderate amount of variably as indicated by large standard error values. Results were similar and followed the same pattern as those observed by Cass-Calay and Bahnick (2002), who reported that no clear trends were evident in commercial catch during 1990 to 2001. Although yellowedge grouper CPUE was not significantly different between the two regions results could have been confounded by the large number of zeros in the data set; yellowedge grouper were only collected at 15.2% of stations.

Comparisons of presence or absence between Zones were significantly different and indicated that yellowedge grouper were more abundant in Zones W and C than in Zone E. Even though CPUE did not differ throughout the GOM, significant differences between positive captures, lengths, weights, ages and density occurred between the regions. Yellowedge grouper were larger, heavier and older in the western GOM (Zone W) followed by the central GOM (Zone C). The eastern GOM (Zone E) had the smallest, lightest and youngest fish. However, yellowedge grouper were found in greatest density in the eastern GOM as opposed to the central and western GOM.

These differences could be a direct result of greater fishing pressure in Zone E primarily off the southwest Florida coast. The largest percentage (44.6%) of commercial longline landings during 1999 to 2004 occurred in Zones E followed by W (34.3%) and C (21.2%) (Personal communication from the National Marine Fisheries Service, Southeast

Fisheries Science Center, Miami, FL). Commercial effort also indicated that yellowedge grouper may be less abundant in Zone E. The majority of commercial longline trips during the same time period occurred in Zone E (70.5%) with near equal effort in Zones W (14.8%) and C (14.7%) (Personal communication from the National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, FL). It took considerably more effort in Zone E to catch yellowedge grouper than in the rest of the GOM.

Although only limited historic data was available, Roe (1976) reported similar results regarding yellowedge grouper collected from 1950-1975; fish were more abundant in the western GOM followed by the central GOM then eastern GOM. Weight data available for twenty-two fish indicated yellowedge grouper were also heavier in the western GOM (mean=5.45 kg) than central GOM (mean=3.95 kg) (no data was available from the eastern GOM) (Roe, 1976). Although mean weights observed by Roe (1976) were lower than those reported in this study, they were probably influenced by small sample size. Currently, yellowedge grouper in the eastern GOM (Zones E and C) were an average of 120 mm smaller and six years younger than those from the western GOM. Yellowedge grouper from Zone E were similar in length to fish from Zone C, but the average age of Zone E fish was slightly older. However, results could be influenced by sample sizes between the Zones and the lack of both young and old fish in Zone E and older fish in Zone C. Commercial samples indicate that yellowedge grouper of all age classes were found throughout the entire GOM. Regional comparisons between other grouper species are not available, however, other species such as red snapper (Allman et al., 2002) and vermilion snapper (Allman et al., 2005b) have also been observed as older and larger in the western GOM. Although historic sample size was small (Roe, 1976) it

does suggest that differences between areas in the GOM existed prior to the expansion of commercial longlining (Bullock et al., 1996) and could simply be natural regional differences in the stocks as opposed to changes in response to fishing pressure. Additional scientific samples which include all age classes in all regions are recommended to determine if actual regional differences exist.

One reason for differences in density between Zones may be due to locations of suitable habitat. The eastern GOM is primarily carbonate substrate possibly making burrow construction difficult due to a lack of cohesive sediment. Areas with the highest densities of yellowedge grouper are from south of Tampa to south of Charlotte Harbor, Florida along the 100 fathom contour. This area is primarily composed of foraminiferal sand and silts (Gould and Steward, 1953) with a thin layer of detrial sediments (Moe, 1963). Rock ridges occur parallel to the 100 fathom contour (Moe, 1963). Therefore yellowedge grouper appear to be associated with smaller reef and rock patches, outcroppings, sinkholes, pockmarks and ledges. This habitat is found in patches, (Moe, 1963) possibly causing yellowedge grouper to live in denser groups to take advantage of the available habitat. This could cause factors such as competition over available food to influence fish size. Competition may be lessened in the western GOM since yellowedge grouper occur over a wider area. Jones et al. (1989) noted that most burrows in the western GOM contained only a single yellowedge grouper possibly indicating less direct competition between individuals.

The objectives of this research were to determine the distribution of yellowedge grouper in the GOM and to evaluate the possibility of separate stocks in the northern GOM. Yellowedge grouper are distributed throughout the GOM; however, regional

differences in both abundance and density were observed. It appears that two or possibly three separate stocks of yellowedge grouper may exist in the GOM. Age, length frequency and weight differences as well as differences in density support the idea that yellowedge grouper are not a single stock. Yellowedge grouper in the western GOM are larger, older and more abundant while fish in the eastern GOM are smaller and younger. Yellowedge grouper in the eastern GOM cluster in denser patches than those in the western GOM. Historical information suggests that differences may naturally occur in the GOM and may not be a result of greater fishing pressure in the eastern GOM. Although yellowedge grouper is currently managed as a single stock in the GOM, multiple stock management strategies may need to be applied to properly manage this species. Since the majority of effort and landings occur in the southeastern GOM where yellowedge grouper display a smaller size and age, managers should closely observe this stock in order to avoid overfishing.

CHAPTER IV

YIELD-PER-RECRUIT AND SPAWNING STOCK BIOMASS-PER-RECRUIT

Introduction

Yellowedge grouper, *Epinephelus flavolimbatus*, are managed in the Reef Fish Resources of the Gulf of Mexico Fisheries Management Plan (RFRFMP). The RFRFMP currently consists of 40 reef fish species (Appendix A) including grouper (Serranidae, *n*=15), snapper (Lutjanidae, *n*=14), tilefish (Malacanthidae, *n*=5), jacks (Carangidae, *n*=4) and also hogfish (Labridae) and gray triggerfish (Balistidae). The RFRFMP is composed of nearly thirty amendments which are directed at individual species, rebuilding plans, fishing practices (bag limits and quotas) and various other fisheries issues. Yellowedge grouper are managed as part of the deep-water grouper complex which also includes snowy grouper, *Epinephelus niveatus*, Warsaw grouper, *Epinephelus nigritus*, misty grouper, *Epinephelus mystacinus*, and speckled hind, *Epinephelus drummondhayi* (GMFMC, 2001). Current Gulf of Mexico (GOM) management regulations include an annual 1.02 million pound gutted weight deep-water grouper quota and no minimum size limits for commercial fishermen (GMFMC, 2005 a). Recreational fishers can harvest five grouper per-person daily in an aggregate of all grouper species and there are no minimum size limits for yellowedge grouper (GMFMC, 2005 b).

The National Marine Fisheries Service (NMFS) is required by the Magnuson-Stevens Fishery Conservation and Management Act (MSFCMA) "to conserve and manage the fishery resources found off the coasts of the United States" (NMFS, 1996). The National Standard Guidelines for the MSFCMA require the NMFS to establish definitions of overfishing and the condition of being overfished for each species listed in

a fishery management plan (FMP). A maximum fishing mortality threshold must also be established and the status of stocks is to be evaluated (NMFS, 1996; DOC, 1998).

Biological reference points are used by fisheries managers to assess different aspects of fishing on the status of a stock. Reference points are used to evaluate if a stock is "overfished" or undergoing "overfishing" and to provide guidelines for harvest. Overfishing reference points are broken down into target and limit points. Managers aspire toward target points which aim toward a sustainable stock. The goal of management is to avoid strategies that approach or exceed limit reference points. Limit points indicate levels where either fishing mortality or stock biomass could result in a stock collapse (Gabriel and Mace, 1999). Two different types of overfishing can be assessed, growth and recruitment. Growth overfishing occurs when fish are harvested before they can attain an optimal size. This leads to smaller size and younger fish being harvested (NMFS, 2006). Recruitment overfishing occurs when the rate of fishing significantly reduces the annual recruitment of the exploitable stock. The spawning stock is reduced resulting in the harvest of fish before they have a chance to reproduce and the loss of older individuals from the population. If the stock is depleted to a point where it cannot replace those fish that are harvested the stock will eventually collapse (Haddon, 2001; NMFS, 2006).

Dynamic pool models such as yield-per-recruit (YPR) and spawning stock biomass-per-recruit (SSB/R) are commonly used as a means to determine biological reference points (Thompson and Bell, 1934; Beverton and Holt, 1957). Dynamic pool models incorporate biological processes such as growth, vulnerability by age to fishing, reproductive potential and mortality (either due to fishing (F) or natural causes (M)).

They are used to evaluate the effects of various levels of fishing effort on the yield and spawning stock of a single cohort expressed on a per-recruit scale. Recruits (fish newly vulnerable to exploitation) are either harvested and contribute as yield or they remain in the population and contribute as a member of the spawning stock (NMFS, 2006).

Yield-per-recruit models are used to evaluate growth overfishing and determine several important biological reference points. These models can be used to determine a target fishing mortality, minimum age at first capture and definition of growth overfishing. Yield-per-recruit reference points include F_{Max} and $F_{0.1}$. F_{Max} is the limit reference point that describes the fishing mortality rate which corresponds to the maximum yield produced by a recruit (Gabriel and Mace, 1999). $F_{0.1}$ was developed for use as a target reference point, and is determined by calculating the fishing mortality rate at which the slope of the YPR curve is 10% of the slope at the origin (NMFS, 2006). The value of $F_{0.1}$ is an arbitrary value that reflects a reduction in effort without a significant reduction in yield (Gulland and Borema, 1973). Growth overfishing occurs when the fishing mortality rate (F) exceeds F_{Max} which results in a reduction in yield regardless of increased effort (Haddon, 2001; NMFS, 2006). However, fishing at optimal F values predicted by YPR analysis does not guarantee the sustainability of a stock which is why SSB/R models are often integrated with YPR analyses.

Spawning stock biomass-per-recruit models are an extension of YPR used to define recruitment overfishing by evaluating the effects of fishing mortality on the spawning potential of a stock (Gabriel et al., 1989). These models evaluate the expected lifetime contribution to the spawning stock biomass by the average recruit (NMFS, 2006). Spawning stock biomass is defined as the total weight of both males and females

in the population that contribute to reproduction (NMFS, 2006). The current overfishing threshold for all reef fish managed under the RFRFMP (excluding red snapper, *Lutjanus campechanus*, Nassau grouper, *Epinephelus striatus*, and goliath grouper, *Epinephelus itajara*) is a fishing mortality rate equivalent to 30% static spawning potential ratio (SPR, $F_{30\%\ SPR}$; GMFMC, 1999). Spawning potential ratio is a measure of the female reproductive capability of the stock defined as the number of eggs that could be produced by an average mature recruit during its lifetime in a fished stock, divided by the number of eggs that could be produced by an average recruit in an unfished stock (NMFS, 2006). Spawning potential ratio varies from one and declines to zero as F increases. Spawning potential is often measured as fecundity-per-recruit; however, in the absence of fecundity information SSB/R in a fished and unfished state is an appropriate substitute (Goodyear, 1993; NMFS, 2006). Spawning stock biomass-per-recruit reference points are the fishing mortality rate at $F_{xx\%\ SPR}$ ($F_{30\%\ SPR}$ for most GOM reef fish) which is used to define recruitment overfishing and the maximum spawning potential (MSP) which is the SSB/R at F_{zero} (no fishing mortality) which is used to calculate SRP. The effect of fishing on SSB/R is expressed as percent MSP (F_{zero}=100% MSP) and illustrates the reduction of spawning potential with the increase of fishing mortality (NMFS, 2006). In the GOM, F_{MSY} (the fishing mortality rate that, if applied constantly, resulted in maximum sustainable yield) is equivalent to $F_{30\%\ SPR}$ for most reef fish stocks (GMFMC, 1999).

Yield-per-recruit models have been successfully used to assess the status of numerous reef fish species in the western Atlantic Ocean such as red snapper, vermilion snapper, speckled hind, gag, scamp and snowy grouper (Huntsman et al., 1983; Matheson et al., 1984; Huntsman et al., 1994). Sadovy and Figuerola (1992) used a YPR model to

assess red hind, *Epinephelus guttatus*, in Puerto Rico and St. Thomas. Yield-per-recruit and SSB/R models were widely applied to groundfish and flounder stocks in the northwestern Atlantic (Gabriel et al., 1989; Working group on re-evaluation of biological reference points for New England groundfish, 2002; Cadrin and King, 2003; Wigley and Burnett, 2003; Terceiro, 2006).

The first stock assessment on yellowedge grouper in the northern GOM was conducted in 2002 using a state-spaced age structured production model (Cass-Calay and Bahnick, 2002; Porch, 2003). Due to a lack of available life history information and the short catch-per-unit-effort (CPUE) and landings time series (1985-2001) the assessment was inconclusive and the population dynamics remained unknown (Cass-Calay and Bahnick, 2002). The objectives of this research were to apply YPR and SSB/R models to yellowedge grouper life history and fishery data to provide biological reference points that were previously unavailable. In addition, the alternate hypotheses that yellowedge grouper are undergoing growth overfishing and recruitment overfishing were also evaluated. Male SPR, as well as the traditional female SPR, was examined given that it is important yet overlooked information that should be assessed when managing a hermaphroditic species. Finally, results were used to provide both insight regarding the status of the stock and recommendations for future management.

Methodology

Yellowedge grouper collected during 1999-2005 on fishery independent bottom longline research cruises and by the commercial bottom longline fishery were used to calculate parameters used in the YPR and SSB/R analyses. Yellowedge grouper collected during that time by the NMFS using trawling gear were excluded given that

there is no commercial trawl fishery for yellowedge grouper. Also, fish collected using commercial hand lines were excluded due to significant differences in size and age between longline and hand line fisheries (see Chapter II). A lack of samples prevented a separate analysis for the commercial hand line fishery. Since no significant difference in total length or age was found between fishery independent and dependent sectors (see Chapter II) all data were combined for the analysis. Several parameters used in the YPR and SSB/R models were calculated prior to running the models. Initially, YPR and SSB/R analyses were to be conducted by region, eastern and western GOM, since evidence existed that at least two different stocks were present in the GOM (see Chapters II and III). However, a lack of samples from the western GOM prevented accurate calculations of reproductive parameters and recruitment into the fishery. Therefore, data from both regions were combined for the analyses.

Growth

The length-weight relationship was computed using yellowedge grouper collected where both weight and length data were available. The relation between length and weight is described as a power function:

$$W = aL^b$$

where:

W = total fish weight (kg) and;

L = total length (mm)

b is the allometric growth parameter which typically ranges from 2.7-3.5 (Slipke and Maceina, 2001) and a is a constant. Results from the 1999-2005 von Bertalanffy growth

equation (see Chapter II for details) and weight-length relationship were used to construct the von Bertalanffy growth equation for body weight:

$$W_t = W_\infty \left[1 - e^{-K[t-t_0]}\right]^b$$

where:

W_∞ = asymptotic weight of a fish;

K = growth coefficient from the von Bertalanffy growth equations;

t_0 = theoretical age at length zero;

b = the allometric growth parameter from the weight-length relationship and;

e=exponent for natural logarithms.

Least-squares non-linear regression analysis was used to determine the value of W_∞. The von Bertalanffy weight equation was used to calculate weight at age for each age group.

Selectivity

Simulations were conducted using gradual recruitment of an age group to the fishery as opposed to knife-edge recruitment (entire age group becomes vulnerable at the same time). Determination of proportion of fish per age group (t) vulnerable to fishing was determined in two steps (Huntsman et al., 1983). The minimum length yellowedge grouper recruited to the fishery (l_r) was determined by first grouping fish into 50 mm total length (TL) size intervals, selecting the first size interval which contained five or more fish, and then calculating the mean TL of the interval (Huntsman et al., 1983). Next, the probability that a yellowedge grouper of age t was greater than or equal to l_r was determined for each age group based on the assumption of a normal distribution of total lengths about the mean length at age t (Huntsman et al., 1983). A Kolmogorov-Smirnov one-sample test was first used to confirm that the TL of all age groups was

normally distributed around the mean (P>0.05 for all groups). The first age group with a probability > 0.99 was considered the first age group to be completely vulnerable to the fishery (Huntsman et al., 1983).

Total (Z), Natural (M) and Fishing (F) Instantaneous Mortality Rates

Total mortality (Z) was calculated using a catch curve analysis (Edser, 1908; Robson and Chapman, 1961). The catch curve analysis used the age frequency distribution of all yellowedge grouper fully recruited into the fishery to determine Z. The natural log of total number of fish at age was regressed against age, and Z is negative times the slope of the regression equation. The slope is negative, therefore, making Z a positive number. A catch curve was constructed using yellowedge grouper collected during 1999-2005 by fishery independent and dependent bottom longline gear. Catch curves were also constructed for time periods 1979-1989 and 1991-1992 using all yellowedge grouper collected (including NMFS trawl and hand line collected fish) to allow for enough samples per age group to complete the analysis. The additional catch curves were used merely to observe if changes in Z occurred over time. Time periods were selected based on natural breaks in the sample collections (see Chapter II).

Ideally, natural mortality (M) should be calculated before the onset of fishing, however, this rarely occurs. Due to the difficulty estimating M multiple formulas were used:

Hoenig (1983):

$$\ln(M) = 1.46 - 1.01 * \ln(t_{\max})$$

where:

t_{max} = maximum age of fish.

Jensen (1996):

$$M = 1.50 * |K|$$

where:

K = von Bertalanffy growth coefficient.

Pauly (1980):

$$\log_{10}(M) = -0.0066 - 0.279 * \log_{10}(L_\infty) + 0.643 * \log_{10}(K) + 0.4634 * \log_{10}(\text{TEMP})$$

where:

K = von Bertalanffy growth coefficient;

L_∞ = von Bertalanffy asymptotic length of a fish;

TEMP = annual water temperature.

Annual water temperature used was 18.5°C, the mean bottom water temperature where yellowedge grouper were captured, determined using environmental data collected on NMFS scientific cruises. Natural mortality was calculated for each time period and using all data combined in order to establish the best estimate of M.

Instantaneous fishing mortality (F) was calculated using estimates of Z and M by the formula:

$F = Z - M$.

Yield-per-recruit and spawning stock biomass-per-recruit models

Both YPR and SSB/R models were constructed using NOAA Fisheries Toolbox (NFT) version 2.6.2 software (NFT, 2006). The yield and biomass per-recruit models were based on a modified Thompson Bell model (Thompson and Bell, 1934) as described by Gabriel et al. (1989). The models assumed steady-state conditions, partial recruitment and mean weight at age for yellowedge grouper collected from 1999-2005. The YPR and

SSB/R models are sensitive to estimates of natural mortality; therefore, a sensitivity analysis was conducted using estimates of *M* ranging from 0.040-0.090 using intervals of 0.005 to determine the effect *M* had on F_{Max}, $F_{0.1}$ and $F_{30\% SPR}$.

Input data required for the YPR and SSB/R simulations included the following parameters. (1) Weight at age data for stock weights, catch weights and spawning stock weights were calculated using the von Bertalanffy weight equation. All weights were considered the same for each group since no previous research was available to indicate that weight varied by group. (2) Selectivity on *F* (as described above) and *M* per age group. The selectivity on *M* was assumed to be the same (1.0) for all age groups since no previous research was available to indicate that *M* varied by age group. (3) The SSB/R model required input on proportion of both *F* and *M* before the onset of spawning in order to account for the mortality that occurred before the opportunity to spawn. Since yellowedge grouper have a prolonged spawning season, a value 0.5 which represented July 1 (the start of the peak spawning season) was used for both *F* and *M*. (4) The original SSB/R model was designed for gonochoristic species with a 1:1 sex ratio, therefore, additional calculations were made to account for a hermaphroditic species with an unequal sex ratio. The fraction of sexually mature individuals was calculated for females based on an age at maturity ogive. Since all males are sexually mature, the fraction of males in the population was based on the proportion of males at age. Separate SSB/R analyses were conducted for both males and females. (5) The number of age groups included was based on 3/*M* where *M*=0.048 (the lowest estimate calculated for *M*) which corresponded to ages 1-63 years old (Anthony, 1982; Gabriel et al., 1989).

Results

Growth

The relationship between yellowedge grouper weight-length was described as $weight = 0.0000000278 * total\ length^{2.867}$; $n=635$; $R^2=0.984$ (Figure 42).

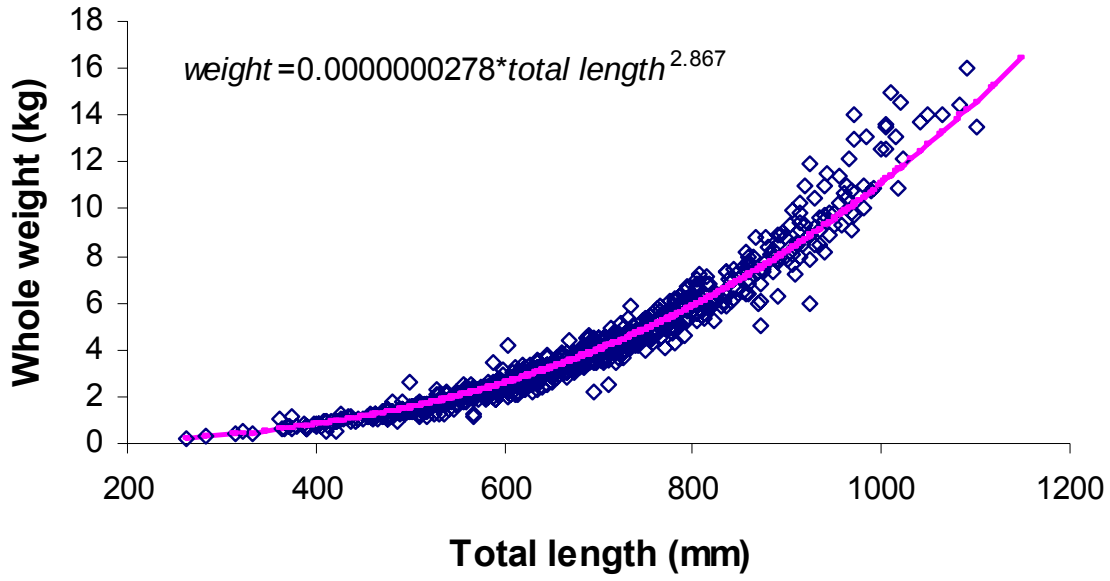

Figure 42. Yellowedge grouper weight-length relationship. Yellowedge grouper were collected during 1999-2005 using bottom longline gear.

The von Bertalanffy growth equation for body weight was described as $W_t = 12.95\left[1 - e^{-0.063[t-(-3.996)]}\right]^{2.867}$ where W_∞=12.95 kg (Figure 43). Body weight increased sharply for the first thirty years then leveled off as it approached the asymptote.

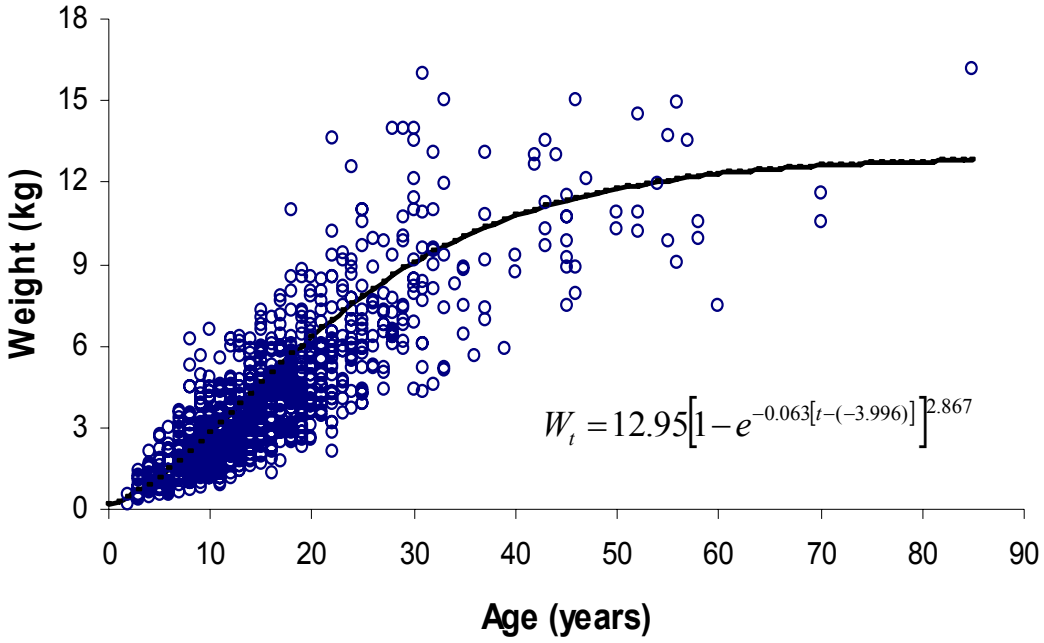

Figure 43. Yellowedge grouper weight-age relationship. Yellowedge grouper were collected during 1999-2005 using bottom longline gear. Weight is represented as *W* and *t* represents age.

Selectivity

The minimum length yellowedge grouper recruited to the fishery (l_r) was 380 mm TL and two years of age. Partial recruitment was observed beginning at age two when 2% of yellowedge grouper were vulnerable to the fishery. Recruitment increased considerably to 62% by age three, 78% by age four and by age seven 99% of fish were susceptible to fishing (Table 17). At eight years of age all yellowedge grouper were fully recruited to the fishery.

Table 17. Yellowedge grouper recruitment to the bottom longline fishery. Sample size is defined by *n*, TL refers to total length, minimum and maximum refer to the range of total lengths observed in each age class, standard (Std.) error and deviation, and proportion vulnerable to the fishery.

Age (years)	*n*	Minimum TL (mm)	Maximum TL (mm)	Mean TL (mm)	Std. Error	Std. Deviation	Proportion vulnerable	*Z*-score
2	2	262	322	292.00	30.00	42.43	0.02	2.074
3	10	283	486.00	399.80	19.66	62.19	0.62	-0.318
4	16	315	532.00	426.44	15.27	61.10	0.78	-0.760
5	16	373	621.00	468.75	18.44	73.74	0.89	-1.204
6	21	363	635.00	484.62	17.56	80.45	0.90	-1.300
7	30	374	695.00	553.77	14.35	78.62	0.99	-2.210

Total (Z), Natural (M) and Fishing (F) Instantaneous Mortality Rates

The catch curve analysis indicated that *Z* has increased over time (Figure 44; Table 18) and the most recent estimate was approximated as Z=0.128 based on yellowedge grouper collected during 1999-2005. The age classes used to construct the catch curves varied per time period due to the age at which all fish were fully recruited into the fishery and the number of samples at older age classes. The lack of samples, particularly in the younger age classes, and combination of bottom longline and hand line gear during 1979-1989 and 1991-1992 possibly influenced the estimate of age at full recruitment into the fishery and the estimate of *Z*. However, the combination of different gear types used to collect yellowedge grouper was necessary to obtain enough samples to construct the catch curve.

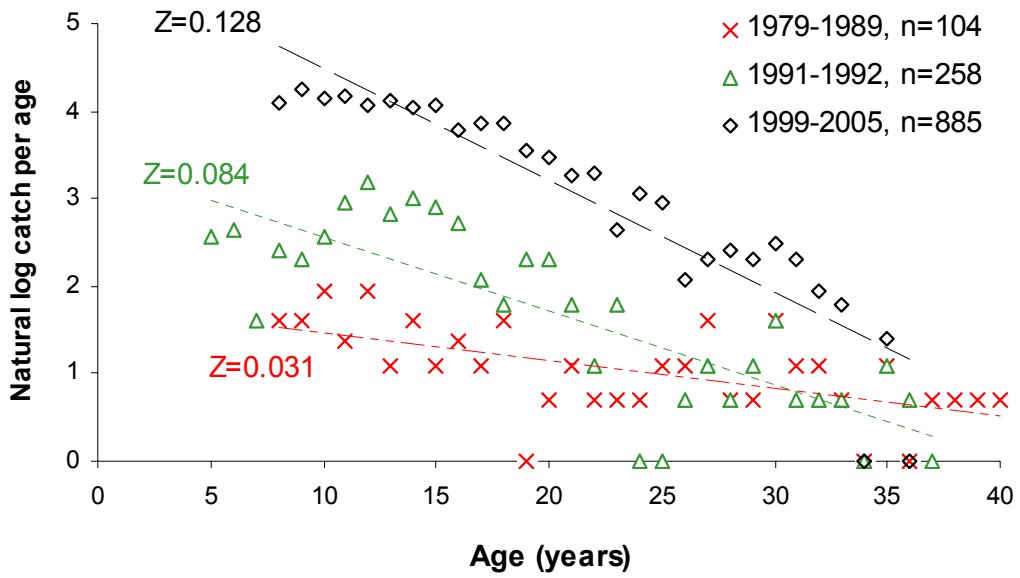

Figure 44. Yellowedge grouper catch curve analysis over time. Yellowedge grouper were collected using bottom longline and hand line gear during 1979-1989 and 1991-1992 and using only bottom longline gear during 1999-2005.

Table 18. Yellowedge grouper catch curve regression analysis results. Ages refer to the number of age classes used in the analysis, *n* is the sample size, *df* is the degrees of freedom, SS is the sums of squares, *P* is the probability, and *Z* is the instantaneous total fishing mortality. Time periods 1979-1989 and 1991-1992 included a combination of bottom longline gear and hand line gear used for yellowedge grouper capture, only bottom longline gear was used during 1999-2005.

Time period	Ages (years)	*n*	Source	*df*	SS	Mean square	*F*-value	*P*	Intercept	*Z*	R^2
1979-1989	8-40	104	Model	1	2.96	2.96	16.83	0.001	1.78	0.031	0.35
			Error	31	5.46	0.18					
			Total	32	8.42						
1991-1992	5-37	285	Model	1	21.20	21.20	56.67	0.001	3.40	0.084	0.66
			Error	31	11.01	0.36					
			Total	32	32.21						
1999-2005	8-36	885	Model	1	33.07	33.07	151.42	0.001	5.76	0.128	0.85
			Error	27	5.90	0.22					
			Total	28	38.97						

Predicted *M* ranged from 0.048-0.174 depending on method used (Table 19). Estimates of *M* during 1999-2005 were 0.048, 0.090 and 0.174 based on the equation used (Hoenig, 1983; Jensen, 1996; Pauly, 1980, respectively). The estimate of *M* using Pauly (1980) exceeded that of *Z* in all cases and was considered inaccurate and not used in further analyses. Fishing mortality during 1999-2005 was estimated as F=0.080 or F=0.038 based on calculations of M=0.048 or M=0.090, respectively.

Table 19. Predicted rates of yellowedge grouper natural mortality (*M*) derived from three methods. Methods included 1) Hoenig (1983), 2) Jensen (1996) and 3) Pauly (1980). *K* represents the von Bertalanffy growth coefficient, t_{max} is the maximum age of the fish during the corresponding time period, L_∞ is the von Bertalanffy asymptotic length (mm) and Temp is the overall mean bottom water temperature where yellowedge grouper were collected.

Time period	*K*	L_∞ (mm)	t_{max}	Temp (°C)	Method 1 (*M*)	Method 2 (*M*)	Method 3 (*M*)
1979-1989	0.042	966.9	81	18.5	0.051	0.057	0.134
1991-1992	0.058	974.6	77	18.5	0.054	0.048	0.165
1999-2005	0.063	995.5	85	18.5	0.048	0.090	0.173
All data	0.063	970.8	85	18.5	0.048	0.086	0.174

Yield-per-recruit and spawning stock biomass-per-recruit models

Input data used to construct the YPR and SSB/R analyses are presented in Appendix B. Yield, and both female and male spawning biomass per-recruit were estimated over a range of fishing mortality rates (Appendix C and D) using natural

mortality rates of 0.048 (Figure 45 A) and 0.090 (Figure 45 B). These were the natural mortality estimates determined during 1999-2005 using the formulae developed by Hoenig (1983) and Jensen (1996). Estimates of biological reference points (Table 20) indicated that yellowedge grouper should be subjected to low levels of F in order to sustain the fishery. Yield-per-recruit is not gender specific since yellowedge grouper begin life as females and transform into males. Results demonstrated that at M=0.048 the maximum YPR (1.73 kg) would be achieved at F_{Max}=0.067 and at M=0.090 the maximum YPR (0.97 kg) would occur at F_{Max}=0.124. Fishing effort at $F_{0.1}$=0.041 (when M=0.048) only resulted in a 5.8% loss in yield but produced a 39% reduction in F. Fishing at $F_{0.1}$=0.066 (when M=0.090) resulted in a 7.6% loss in yield but caused a 46% reduction F. Total biomass-per-recruit (the biomass that each recruit contributed to the population) at F_{Max} was 28.8 kg verses 43.0 kg at $F_{0.1}$ when M=0.048. If M=0.090, total biomass-per-recruit was 9.9 kg at F_{Max} and 15.8 kg at $F_{0.1}$.

The pattern of SSB/R was considerably different for females and males as predicted. When M=0.048, at low levels of F males produced a greater SSB/R than females. This is because at low levels of mortality more males lived longer while continuing to reproduce, thus contributing more to the spawning stock. However, as F increased more males were removed from the population and less reached their maximum lifespan. At approximately F=0.05 female SSB/R surpassed male SSB/R. Under the simulation of M=0.090 female SSB/R always exceeded that of males because under higher M even fewer males reached their maximum lifespan. For this reason, females appeared to tolerate a higher level of F based on spawning potential than males. Female $F_{30\%\ SPR}$=0.100 while male $F_{30\%\ SPR}$=0.038 under M=0.048. Estimates of female and male

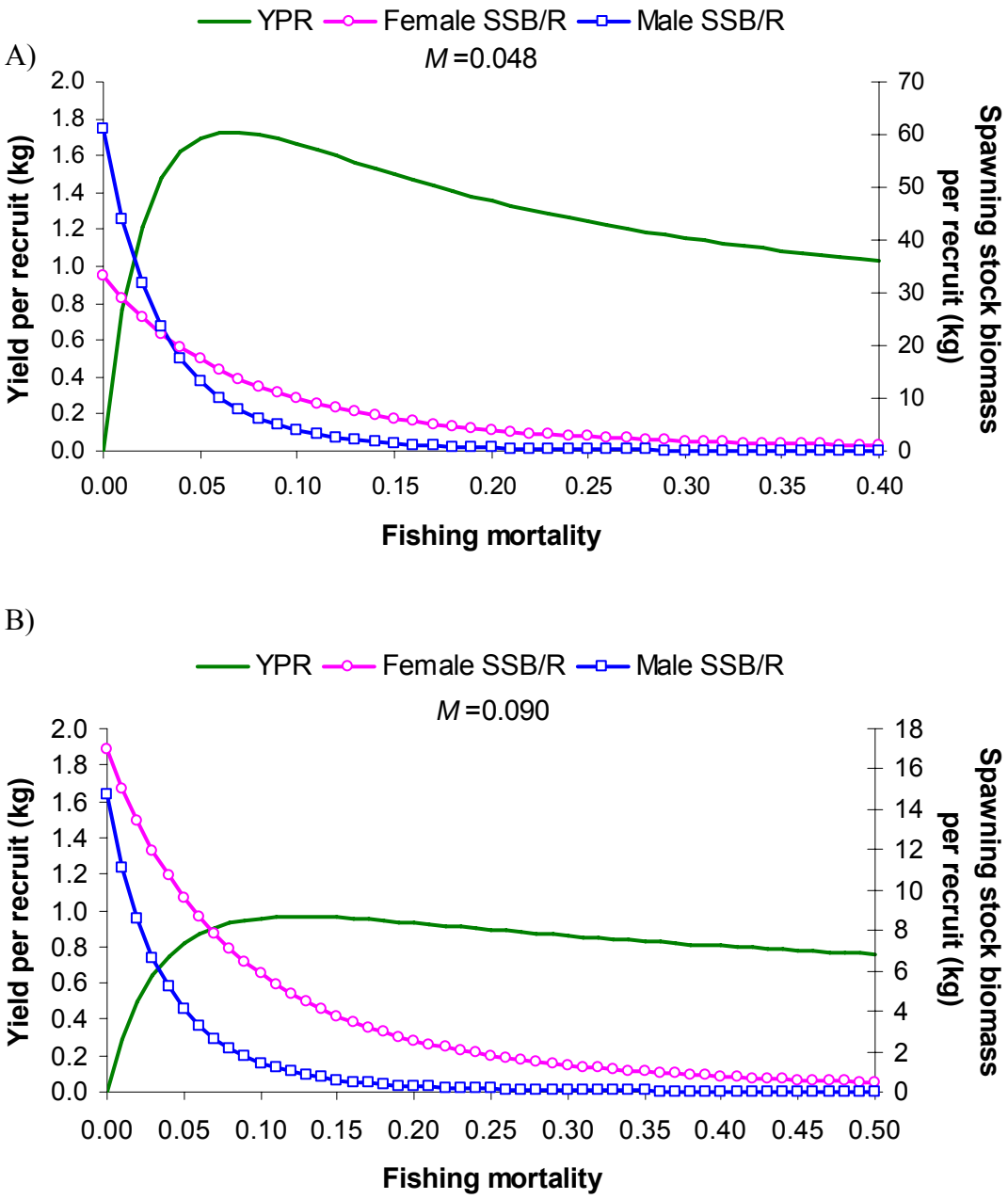

Figure 45. Yellowedge grouper yield-per-recruit (YPR) and spawning stock biomass-per- recruit (SSB/R) calculated for natural mortality (*M*) equals A) 0.048 and B) 0.090.

Table 20. Summary of yellowedge grouper biological reference points as determined by yield-per-recruit (YPR) and spawning stock biomass-per-recruit (SSB/R) models. Reference points were calculated using yellowedge grouper collected using bottom longline gear from 1999-2005.

M	Reference Point	*F*	YPR (kg)	SSB/R (kg)		Total biomass-per-recruit (kg)		Mean age (years)		Mean generation time (years)		Expected spawnings	
				Females	Males	Females	Males	Females	Males	Females	Males	Females	Males
0.048	*F zero*	0.000	0.00	33.11	61.19	101.89	101.89	18.12	18.12	17.23	37.45	7.55	6.45
	$F_{0.1}$	0.041	1.63	19.37	17.53	42.96	42.96	11.71	11.71	15.62	31.79	4.87	2.01
	F_{Max}	0.067	1.73	14.28	8.53	28.80	28.80	9.48	9.48	14.73	28.81	3.80	1.08
	$F_{30\% \text{ SPR}}$ Females	0.100	1.66	9.94		19.24		7.66		13.72		2.83	
	$F_{30\% \text{ SPR}}$ Males	0.038	1.60		18.37		44.98		12.00		32.12		2.15
0.090	*F zero*	0.000	0.00	16.99	14.69	37.58	37.58	11.40	11.40	15.58	31.66	4.28	1.73
	$F_{0.1}$	0.066	0.89	8.09	2.84	15.76	15.77	7.16	7.16	13.47	24.91	2.30	0.40
	F_{Max}	0.124	0.97	4.70	0.93	9.86	9.86	5.61	5.61	12.06	20.94	1.51	0.15
	$F_{30\% \text{ SPR}}$ Females	0.115	0.96	5.10		15.52		5.80		12.27		1.61	
	$F_{30\% \text{ SPR}}$ Males	0.047	0.80		4.41		19.43		8.00		26.61		0.59

$F_{30\%\ SPR}$ when M=0.090 were 0.115 and 0.047, respectively. The spawning potential of males quickly reduced as F increased (Figure 46). At $F_{30\%\ SPR}$ for males, female maximum spawning potential was only reduced by roughly 60% regardless of M used.

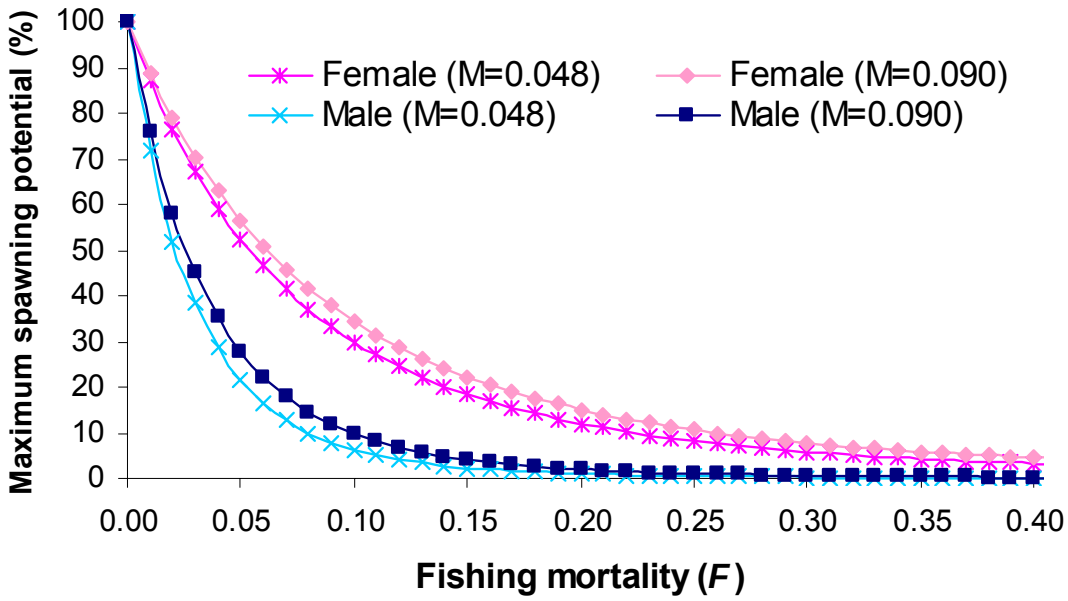

Figure 46. Effects of fishing mortality (F) on female and male spawning potential when natural mortality (M) was equal to 0.048 and 0.090.

Sensitivity analyses illustrated how variable YPR, SSB/R and biological reference points were depending on M used (Figure 47). Higher levels of natural mortality resulted in biological reference points with higher levels of fishing mortality. Estimates of F_{Max} and $F_{0.1}$ were more sensitive to M than $F_{30\%\ SPR}$. When M=0.090 the estimated F_{Max} and $F_{0.1}$ were nearly double that of M=0.040. Female and male $F_{30\%\ SPR}$ only differed by 15.3% and 21.3%, respectively, at the extreme estimates of M. SSB/R reference points

were less sensitive to estimates of M because they are expressed as a percent of the maximum spawning potential (e.g., $F_{30\%SPR}$), and natural mortality affects both the unfished and fished population. Conversely, YPR reference points are not a percentage or related to an unfished condition, therefore, M has a greater effect on them.

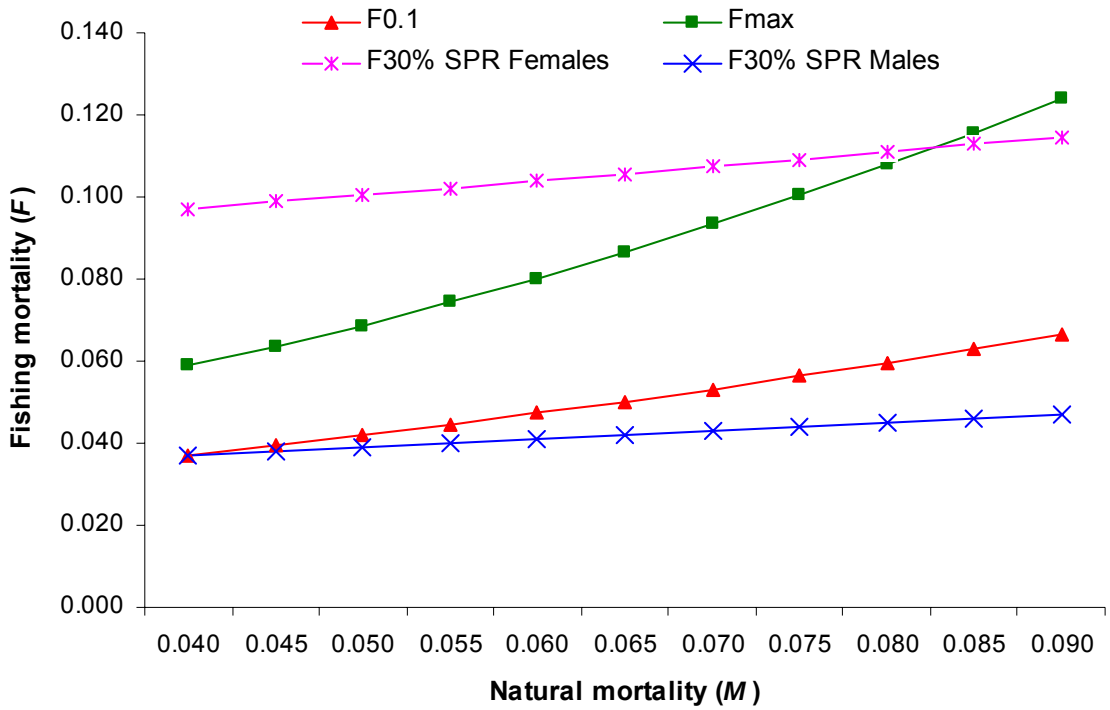

Figure 47. Results of sensitivity analyses on the influence of natural mortality (M) on the calculation of biological reference points. SPR refers to spawning potential ratio.

Under the current estimate of Z=0.128 if natural mortality was equal to 0.048 yellowedge grouper are experiencing growth overfishing since F (0.080) was greater than F_{Max} (0.067). However, yellowedge grouper are not undergoing recruitment overfishing since F was less than $F_{30\%\ SPR}$ (0.100). Currently, female SPR was estimated as 0.37.

However, although not used to define recruitment overfishing male SPR was 0.10 when M=0.048, which equates to only 10% of the virgin male spawning stock remaining.

Yellowedge grouper are not experiencing growth or recruitment overfishing as defined by the criteria of YPR and female $F_{30\% \text{ SPR}}$ when M=0.090 was used to evaluate the stock. The estimated F (0.038) suggested that fishing effort was considerably less than both $F_{0.1}$ (0.066) and F_{Max} (0.124). Female SPR was estimated as 0.63 and male SPR was 0.35.

Discussion

The value of population modeling relies on the accuracy of parameter estimates, relationships and assumptions used. Both YPR and SSB/R models are sensitive to estimates of M and are influenced by the growth rate and longevity of a species (Huntsman et al., 1983). Yield-per-recruit and SSB/R assume that the age structure of the population is in equilibrium and that exploitation, growth and M are constant over the life span of a year class (NMFS, 2006), assumptions that are often violated. The most accurate method to estimate M is to survey an unfished population and construct a catch curve analysis since all mortality is from natural causes; therefore, Z would be equal to M. However, it is often impossible to find an unfished population. This method was used by Moore and Labisky (1984) to derive an estimate of M=0.175 for an unfished population of snowy grouper in the Florida Keys. Although snowy grouper are a closely related deep-water grouper they do not appear to live as long as yellowedge grouper and as a result may have different natural mortality rates. The oldest snowy grouper reported by Moore and Labisky (1984) was 27 years old which is consistent with other maximum ages reported in the South Atlantic Bight (Matheson and Huntsman, 1984) and off the

Carolinas (Wyanski et al., 2000). Therefore, an estimate of snowy grouper natural mortality is probably not an appropriate substitute to use for yellowedge grouper natural mortality. Unfortunately yellowedge grouper biological sampling did not begin until after the onset of exploitation and unfished populations are no longer believed to exist.

Use of multiple methods to estimate *M* in a single study is typical due to the uncertainty in the calculation of *M*. Gunderson et al. (2003) used methods described in Hoenig (1983), Jensen (1996) and Pauly (1980) to approximate *M* for arrowtooth flounder, *Atheresthes stomias*, and darkblotched rockfish, *Sebastes crameri*. Huntsman and Schaaf (1994) used Hoenig (1983) and Pauly (1980) to estimate *M* for graysby, *Epinephelus cruentata*. Estimates of yellowedge grouper natural mortality were 0.048, 0.090 and 0.174 based on the equation used (Hoenig, 1983; Jensen, 1996; Pauly, 1980, respectively). Although the Pauly (1980) method may be appropriate to use for some species it appeared to overestimate *M* when applied to some reef fish species. Matheson and Huntsman (1984) reported that estimates of *M* using Pauly (1980) exceeded that of *Z* (determined using a catch curve analysis) for mutton snapper. This was also true for yellowedge grouper. Matheson and Huntsman (1984) also reported that the Pauly (1980) estimate of *M* (0.27) appeared high for South Atlantic Bight speckled hind, however, Pauly (1980) was used to estimate snowy grouper natural mortality (*M*=0.15). Huntsman and Schaaf (1994) did not use the estimate of *M* produced using Pauly (1980) for graysby because it also appeared high. Pauly (1980) was developed using tropical fishes which may explain why it was inappropriate for many GOM and Atlantic species.

Cass-Calay and Bahnick (2002) used Hoenig (1983) to initially estimate *M* (0.053) for yellowedge grouper in the GOM. In this study, Hoenig (1983) also appeared

to be the most appropriate method to estimate *M*. Anthony (1982) suggested that in most unfished populations, less than 5% of the cohort lived beyond age 3/*M*. That being true, 3/0.048=63 years, suggesting that in an unfished population 95% of yellowedge grouper were less than 63 years old which seems appropriate for a species that lives 85 years. The same formula applied to Jensen (1996) resulted in 3/.090=33 years indicating a much shorter lifespan and that 0.090 is probably an overestimate of natural mortality. The use of Hoenig (1984) may seem inappropriate since the formula was developed using primarily unfished populations, however, the lifespan of yellowedge grouper does not appear impacted since the maximum ages observed today are similar to those of 25 years ago. Finally, Hoenig (1984) only uses the parameter of maximum age in the calculation, the maximum age of 85 years was validated using ^{14}C, further supporting results from the equation. On the contrary, if yellowedge grouper lived even longer prior to the onset of fishing Hoenig (1984) would underestimate *M*.

Estimates of *M* were considerably lower than that of other grouper found in the GOM and western Atlantic; however, yellowedge grouper maximum age estimates are two to three times older than that of other grouper in those areas. Yellowedge grouper mortality estimates are similar to those of other long-lived, deep-water species such as rockfish and orange roughy, *Hoplostethus atlanticus*, which are often reported to live more than 75 years (Calliet et al., 2001). Natural mortality estimates as low as 0.02 were observed for thornyhead rockfish, *Sebastolobus alascanus* and *S. altivelis* (Pearson and Gunderson et al., 2003) and 0.045-0.064 for orange roughy (Branch, 2001). The long lifespan of yellowedge grouper should translate into dissimilar life history parameters compared to other GOM grouper.

Although estimates of *Z* were calculated for all time periods, those from 1979-1989 and 1991-1992 should be viewed with caution. Data were scarce from both time periods and not uniformly collected throughout the GOM. Few samples were available from 1979-1989 and most of those samples were from the western GOM. Data from 1991-1992 was primarily hand line data from Louisiana and best described that area and fishery. The majority of yellowedge grouper collected during 1999-2005 were from the eastern GOM longline fishery. Since significant size and age differences have been observed between eastern and western GOM yellowedge grouper, direct comparisons of *Z* over time were not appropriate. However, results still suggest the gradual increase of mortality over the last few decades as the fishery developed. Although a large number of fish were sampled during 1999-2005, additional samples primarily over recent years would increase the accuracy of estimates of *Z* and consequently increase the accuracy of estimates of *F*. Ideally, catch curves should be constructed annually and by region, eastern and western GOM, since different levels of fishing pressure occur between the two regions (Personal communication from the National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, FL). The classification of a fish species as experiencing growth or recruitment overfishing is based on accurate estimates of *F*. An assessment using commercial fishing effort and landings could improve the estimates of *F* and further support the findings observed in this study.

It is not unexpected that YPR analysis indicated that yellowedge grouper are experiencing growth overfishing. Age, growth and reproductive research results (see Chapter II) all indicated a decline in the size and age of the population since the onset of commercial fishing, particularly in the eastern GOM. Catch curve analysis and

associated fishing mortality results demonstrated an increasing trend in fishing pressure over time. The increase in fishing pressure on the smaller sized fish was too heavy to allow the fishery to produce its maximum yield-per-recruit resulting in growth overfishing. Currently, management actions are not required if a stock is observed as growth overfished since it normally does not effect a stock's ability to replace itself (GMFMC, 1999).

Although recruitment overfishing is not currently observed, results of both the YPR and SSB/R models indicated that yellowedge grouper stocks cannot sustain high levels of fishing mortality. Huntsman and Schaaf (1994) recommended that most grouper should be subjected to low levels of *F*. The combination of a long lifespan and sexual hermaphroditism may make yellowedge grouper susceptible to overfishing (Coleman et al., 1996; Alonzo and Mangel, 2004; Heppell et al., 2006). Yellowedge grouper begin recruitment to the fishery at the age of two and are fully recruited by the age of eight. The sharp increase in recruitment between the ages of two to three is probably due to the movement of juveniles from shallower water to deeper off-shore waters. Male yellowedge grouper do not appear in the population until the age of eight indicating that all males are vulnerable to the fishery. Females begin reaching sexual maturity by the age of two but are not fully mature until seventeen. Histological analysis indicated that approximately 13% of females entered the fishery before having the opportunity to spawn. In order to sustain the stock, female yellowedge grouper must live long enough to reproduce and enough females must continue to survive to transform into males and continue reproducing. The age of sexual transition begins at approximately 15 years, and by 23 years (95% CI=19-27 years) 50% of females have transformed into

males. Therefore, some yellowedge grouper must live over 15 years to contribute to the spawning stock as both a female and male. Alonzo and Mangel (2004) also recommended that protogynous species be subjected to low levels of *F* to allow some males of all age/size classes to escape fishing and reproduce.

By definition SPR refers only to females and the importance of males is often overlooked (Alonzo and Mangel, 2004; Heppell et al., 2006). However, since yellowedge grouper is a hermaphroditic species with unequal sex ratios as well as a decreasing number of males in the population, examination of male SPR is crucial to stock sustainability. Huntsman and Schaaf (1994) used simulations to examine the impacts of fishing on the protogynous graysby and reported that protogynous species may be more vulnerable to fishing than gonochoristic species due to a greater loss of reproductive capacity as *F* increases. If the number of males significantly decreases due to fishing, a reduction in fertilization rate may occur due to sperm limitation (Alonzo and Mangel, 2004). The possibility of sperm limitation is also influenced by spawning aggregation size. Sperm limitation is believed to be greater for species that spawn in small groups such as yellowedge grouper as opposed to those who form large spawning aggregations (Alonzo and Mangel, 2004).

Compensation to the effects of fishing can occur by either conserving the unfished female:male sex ratio or by altering the onset of transition to conserve the male biomass:cohort fecundity ratio (Huntsman and Schaaf, 1994; Alonzo and Mangel, 2004). However, little compensation was currently observed in yellowedge grouper populations. The current sex ratio has 14% fewer males than were present in 1977-1980 (Bullock et al., 1996) indicating a decrease in the sex ratio. Although the age at transition during that

time was unavailable, the size at transition has only slightly reduced (6 mm) to compensate for fishing mortality (Bullock et al., 1996). Currently there is no information on the percentage of male biomass required to avoid sperm limitation since the idea of sperm limitation is seldom investigated. However, male SSB/R results demonstrated that the maximum spawning potential of males dropped considerably faster than that of females. For example, the current fishing mortality rate of F=0.08 allows for 37% of female spawning potential but only allows 10% male spawning potential. Further investigations should be conducted to determine if a stock can persist with such a limited percent of males available to contribute to reproduction.

Although neither estimate of M resulted in recruitment overfishing, an area of concern was observed with the results presented when using M=0.048. The estimated fishing mortality at $F_{30\%SPR}$ for females was greater than that at F_{Max}. Since F_{Max} is considered a limit reference point, values of sustainable SPR should not exceed the limit. This result could have occurred by two scenarios. Either the estimate of M was too low and a higher estimate of M was more appropriate, or the recommended $F_{30\%SPR}$ (GMFMC, 1999) was too low to sustain the stock. Based on the discussion mentioned earlier, the estimate of M=0.048 seemed appropriate; therefore, $F_{30\%SPR}$ for females may be inappropriate. The reference point of $F_{30\%SPR}$ is not a standard value applied to all fish species in the United States. A value of $F_{40\%SPR}$ was set for Pacific coast rockfish species (Dorn, 2002) and northeastern yellowtail flounder stocks (Cadrin and King, 2003). In the GOM, Nassau grouper and goliath grouper are managed under $F_{50\%SPR}$ (GMFMC, 1999). Dorn (2002) observed that the SPR at maximum sustainable yield varied between $F_{40\%SPR}$ and $F_{60\%SPR}$ for many species of Pacific coast rockfish stocks and that the current $F_{40\%SPR}$

harvest rate exceeded the estimated F_{MSY} for nearly all rockfish stocks. He recommended a conservative SPR harvest rate of $F_{55\%SPR}$ to $F_{60\%SPR}$. It appears that due to the long lifespan of yellowedge grouper a more conservative SPR similar to that of $F_{50\%SPR}$ for Nassau grouper and goliath grouper may be more appropriate. A value of $F_{50\%SPR}$ for females resulted in similar levels of fishing mortality at either estimate of M (F=0.051 when M=0.048; F=0.057 when M=0.090) supporting the higher SPR due to the uncertainties regarding M. However, if $F_{50\%SPR}$ were adopted as the new means used to define overfishing yellowedge grouper would be experiencing recruitment overfishing if M=0.048 was used for the analysis.

Cass-Calay and Bahnick (2002) estimated that F_{MSY} was between 0.050 and 0.076. This recommendation is questionable for females and may be too high for males since it would only leave approximately 22% or 12%, respectively, of the male spawning potential. Although the data used in this study indicated that yellowedge grouper are not currently experiencing recruitment overfishing, the population has been impacted by the effects of fishing resulting in growth overfishing. Presently 95% of yellowedge grouper are less than 33 years of age indicating a considerable loss of older males from the population although the lifespan of the population does not appear truncated. The average age of yellowedge grouper harvested from 1999-2005 was 16 years old. The average age of females and males was 13 and 24 years, respectively. The median ages were similar to the means for both females (median=13 years) and males (median=22 years) indicating that enough yellowedge grouper are currently surviving to reproduce and also transform into males. Although there does not appear to be a great enough loss

of males to significantly impact reproduction, the number of times that a male can contribute to the spawning population has been severely reduced.

Recent management actions may positively contribute to yellowedge grouper, growth, reproduction and spawning stock biomass. The deep-water grouper fishery was closed early for the last three years (July 15, 2004, June 23, 2005 and June 27, 2006) due to fishers meeting the total allowable catch. This should have a positive impact on the stock since peak spawning occurs from July through September, therefore allowing no fishing pressure during the peak of the spawning season. The closure will also allow fish to grow for nearly half the year without exposure to fishing pressure. The impact of the closure will not be realized for several years since most of those offspring have yet to recruit to the fishery. Yellowedge grouper in the northern GOM have experienced population changes due to fishing. However, continued closure through the peak spawning season may compensate for some of the impacts due to fishing.

Results from this study indicated that yellowedge grouper are experiencing growth overfishing but under the current definition are not classified as recruitment overfished. This research demonstrated the first practical application of a SSB/R model applied to a male hermaphroditic population. It illustrated the importance of the male spawning potential and demonstrated the possibilities of sperm limitation and reduced reproduction that can occur from fishing a hermaphroditic species. In the future, it is essential that fisheries managers consider males as well as females when making decisions regarding a protogynous species. The current value of $F_{30\%SPR}$ as a definition of overfishing may need to be increased due to the longevity and spawning potential of yellowedge grouper and further investigation is needed. A reduction in fishing effort to

the target fishing effort of $F_{0.1}$ would not only produce 1.6 kg of YPR but would also avoid recruitment overfishing by retaining 60% of the female spawning stock and probably avoid sperm limitation by conserving 29% of the male spawning stock biomass.

CHAPTER V

SIGNIFICANCE OF RESEARCH

The research and results produced from this study greatly contribute to the understanding of the life history and population dynamics of yellowedge grouper, *Epinephelus flavolimbatus*, in the northern Gulf of Mexico (GOM). Prior to this study, only limited information on yellowedge grouper was known. The NMFS 2002 stock assessment on yellowedge grouper (Cass-Calay and Bahnick, 2002) was inconclusive due to a lack of necessary data. Keener (1984) and Bullock et al. (1996) attempted to age yellowedge grouper from South Carolina and Florida, respectively, with little success and independently concluded that yellowedge grouper otoliths were difficult to interpret. Although Bullock et al. (1996) conducted reproductive research in the eastern GOM they did not investigate fecundity or spawning frequency. Finally, there were no published studies describing the stock structure, distribution, abundance and status of yellowedge in the northern GOM.

This study successfully aged and validated maximum age estimates of yellowedge grouper in the northern GOM. Results indicated that yellowedge grouper are notably slow growing, have a considerably longer lifespan than previously reported and can reach maximum ages of 85 years. Results supported the alternate hypotheses that the length and age structure of fish currently harvested is different than that of the past; yellowedge grouper harvested today are smaller and younger. Significant age and length differences were found between yellowedge grouper collected by the commercial longline and hand line fisheries. However, few samples were available from the hand line fishery and additional sample collection is recommended. Reproductive research indicted that

fishing pressure has impacted the sex ratio of yellowedge grouper supporting the alternate hypothesis that the sex ratio has changed over the last 25 years due to the loss of males from the population.

Yellowedge grouper were distributed throughout the GOM, although not randomly distributed by depth; small, young fish quickly moved into deeper offshore waters and became vulnerable to the fishery. Evidence was observed which supported the alternate hypothesis that multiple stocks may exist in the GOM and requires additional investigation. Differences in population density, size and age structure were found between the eastern and western GOM and as a result may require different management measures for the two regions. Reproductive analysis also supported the existence of multiple stocks in the GOM although only a small number of yellowedge grouper from the western GOM were collected. Additional collection of yellowedge grouper, especially from the western GOM, is strongly advised to determine if the age and reproductive differences observed in this study express regional population differences as well as to determine fecundity estimates.

This study determined key life history parameters and biological reference points (Appendix E) that are a crucial component of fisheries management. Final fishery management decisions and actions in the GOM are determined by the GOM Fisheries Management Council (GMFMC). Stock assessments in the GOM are conducted using the South East Data, Assessment and Review (SEDAR) process which is coordinated by the GMFMC, the NMFS and Interstate Commissions. The SEDAR is comprised of three consecutive workshops: the Data Workshop, Stock Assessment Workshop and Stock Assessment Review Workshop. Development of yield-per-recruit (YPR) and spawning

stock biomass-per-recruit (SSB/R) models and derived reference points are an important contribution to the SEDAR Data Workshop since classic stock assessment techniques rely on life history parameter estimates and established reference points to assess the current status of the stock. Results of this study indicated that yellowedge grouper are currently experiencing growth overfishing but not recruitment overfishing. However, they indicated that yellowedge grouper stocks cannot sustain high levels of fishing mortality. The importance of male spawning stock biomass-per-recruit was demonstrated and is suggested as an additional tool for use by managers to avoid stock collapse when managing a protogynous hermaphrodite. This study is the only current research conducted by the NMFS regarding yellowedge grouper in the GOM. Without these results the next yellowedge grouper stock assessment would most likely have also been inclusive. The methods and results determined in this study need to be considered by the members of the next yellowedge grouper Stock Assessment Workshop, currently scheduled for spring 2010.

APPENDIX A

GULF OF MEXICO REEF FISH FISHERY MANAGEMENT PLAN SPECIES

Snappers - Lutjanidae Family

Queen snapper - *Etelis oculatus*
Schoolmaster - *Lutjanus apodus*
Red snapper - *Lutjanus campechanus*
Gray snapper - *Lutjanus griseus*
Mahogany snapper - *Lutjanus mahogoni*
Silk snapper - *Lutjanus vivanus*
Wenchman - *Pristipomoides aquilonaris*
Vermilion snapper - *Rhomboplites aurorubens*
Mutton snapper - *Lutjanus analis*
Blackfin snapper - *Lutjanus buccanella*
Cubera snapper - *Lutjanus cyanopterus*
Dog snapper - *Lutjanus jocu*
Lane snapper - *Lutjanus synagris*
Yellowtail snapper - *Ocyurus chrysurus*

Groupers - Serranidae Family

Snowy grouper - *Epinephelus niveatus*
Goliath grouper - *Epinephelus itajara*
Warsaw grouper - *Epinephelus nigritus*
Marbled grouper - *Epinephelus inermis*
Rock hind - *Epinephelus adscensionis*
Red hind - *Epinephelus guttatus*
Yellowmouth grouper - *Mycteroperca interstitialis*
Speckled hind - *Epinephelus drummondhayi*
Yellowedge grouper - *Epinephelus flavolimbatus*
Red grouper - *Epinephelus morio*
Misty grouper - *Epinephelus mystacinus*
Black grouper - *Mycteroperca bonaci*
Yellowfin grouper - *Mycteroperca venenosa*
Scamp - *Mycteroperca phenax*
Gag - *Mycteroperca microlepis*

Tilefishes - Malacanthidae (Branchiostegidae) Family

Goldface tilefish - *Caulolatilus crysops*
Anchor tilefish - *Caulolatilus intermedius*
Tilefish - *Lopholatilus chamaeleonticeps*
Blackline tilefish - *Caulolatilus cyanops*
Blueline tilefish - *Caulolatilus microps*

Jacks - Carangidae Family

Greater amberjack - *Seriola dumerili*
Almaco jack - *Seriola rivoliana*
Lesser amberjack - *Seriola fasciata*
Banded rudderfish - *Seriola zonata*

Triggerfishes - Balistidae Family

Gray triggerfish - *Balistes capriscus*

Wrasses - Labridae Family

Hogfish - *Lachnolaimus maximus*

APPENDIX B

YIELD-PER-RECRUIT AND SPAWNING STOCK BIOMASS-PER-RECRUIT INPUT DATA

Age (years)	Selectivity on fishing mortality (*F*)	Selectivity on natural mortality (*M*)	Stock weights (kg)	Catch weights (kg)	Spawning Stock weights (kg)	Fraction mature female	Proportion male
1	0.00	1.00	0.30	0.30	0.30	0.00	0.00
2	0.02	1.00	0.47	0.47	0.47	0.02	0.00
3	0.62	1.00	0.67	0.67	0.67	0.04	0.00
4	0.78	1.00	0.91	0.91	0.91	0.09	0.00
5	0.89	1.00	1.17	1.17	1.17	0.18	0.00
6	0.90	1.00	1.46	1.46	1.46	0.32	0.00
7	0.99	1.00	1.77	1.77	1.77	0.51	0.00
8	1.00	1.00	2.10	2.10	2.10	0.66	0.03
9	1.00	1.00	2.44	2.44	2.44	0.79	0.04
10	1.00	1.00	2.80	2.80	2.80	0.87	0.05
11	1.00	1.00	3.16	3.16	3.16	0.90	0.06
12	1.00	1.00	3.52	3.52	3.52	0.91	0.07
13	1.00	1.00	3.89	3.89	3.89	0.90	0.09
14	1.00	1.00	4.25	4.25	4.25	0.88	0.12
15	1.00	1.00	4.62	4.62	4.62	0.86	0.14
16	1.00	1.00	4.97	4.97	4.97	0.83	0.17
17	1.00	1.00	5.33	5.33	5.33	0.79	0.21
18	1.00	1.00	5.67	5.67	5.67	0.75	0.25
19	1.00	1.00	6.01	6.01	6.01	0.70	0.30
20	1.00	1.00	6.34	6.34	6.34	0.65	0.35
21	1.00	1.00	6.66	6.66	6.66	0.59	0.41
22	1.00	1.00	6.97	6.97	6.97	0.53	0.47
23	1.00	1.00	7.27	7.27	7.27	0.47	0.53
24	1.00	1.00	7.55	7.55	7.55	0.42	0.58
25	1.00	1.00	7.83	7.83	7.83	0.36	0.64
26	1.00	1.00	8.10	8.10	8.10	0.31	0.69
27	1.00	1.00	8.35	8.35	8.35	0.26	0.74
28	1.00	1.00	8.59	8.59	8.59	0.22	0.78
29	1.00	1.00	8.83	8.83	8.83	0.18	0.82
30	1.00	1.00	9.05	9.05	9.05	0.15	0.85
31	1.00	1.00	9.26	9.26	9.26	0.12	0.88
32	1.00	1.00	9.47	9.47	9.47	0.10	0.90
33	1.00	1.00	9.66	9.66	9.66	0.08	0.92
34	1.00	1.00	9.84	9.84	9.84	0.06	0.94
35	1.00	1.00	10.02	10.02	10.02	0.05	0.95
36	1.00	1.00	10.18	10.18	10.18	0.04	0.96
37	1.00	1.00	10.34	10.34	10.34	0.03	0.97
38	1.00	1.00	10.49	10.49	10.49	0.02	0.98
39	1.00	1.00	10.63	10.63	10.63	0.02	0.98
40	1.00	1.00	10.76	10.76	10.76	0.02	0.98
41	1.00	1.00	10.89	10.89	10.89	0.01	0.99

APPENDIX B (CONTINUED)

Age (years)	Selectivity on fishing mortality (*F*)	Selectivity on natural mortality (*M*)	Stock weights (kg)	Catch weights (kg)	Spawning Stock weights (kg)	Fraction mature female	Proportion male
42	1.00	1.00	11.01	11.01	11.01	0.01	0.99
43	1.00	1.00	11.12	11.12	11.12	0.01	0.99
44	1.00	1.00	11.23	11.23	11.23	0.01	0.99
45	1.00	1.00	11.33	11.33	11.33	0.00	1.00
46	1.00	1.00	11.42	11.42	11.42	0.00	1.00
47	1.00	1.00	11.51	11.51	11.51	0.00	1.00
48	1.00	1.00	11.60	11.60	11.60	0.00	1.00
49	1.00	1.00	11.68	11.68	11.68	0.00	1.00
50	1.00	1.00	11.75	11.75	11.75	0.00	1.00
51	1.00	1.00	11.82	11.82	11.82	0.00	1.00
52	1.00	1.00	11.89	11.89	11.89	0.00	1.00
53	1.00	1.00	11.95	11.95	11.95	0.00	1.00
54	1.00	1.00	12.01	12.01	12.01	0.00	1.00
55	1.00	1.00	12.07	12.07	12.07	0.00	1.00
56	1.00	1.00	12.12	12.12	12.12	0.00	1.00
57	1.00	1.00	12.17	12.17	12.17	0.00	1.00
58	1.00	1.00	12.22	12.22	12.22	0.00	1.00
59	1.00	1.00	12.26	12.26	12.26	0.00	1.00
60	1.00	1.00	12.30	12.30	12.30	0.00	1.00
61	1.00	1.00	12.34	12.34	12.34	0.00	1.00
62	1.00	1.00	12.38	12.38	12.38	0.00	1.00
63	1.00	1.00	12.41	12.41	12.41	0.00	1.00

APPENDIX C

RESULTS OF YIELD-PER-RECRUIT (YPR) AND SPAWNING STOCK BIOMASS-PER-RECRUIT (SSB/R) WHEN NATURAL MORTALITY (*M*) EQUALS 0.048. *F* represents fishing mortality, *n* is numbers, F is females and M is males.

F	Catch *n*	YPR (kg)	Stock *n*	Stock weight (kg)	Mean age (years)	Spawning stock numbers		SSB/R (kg)		Spawning potential (%)		Mean generation time (years)		Expected spawnings	
						F	M	F	M	F	M	F	M	F	M
0.00	0.0	0.0	20.3	101.9	18.1	7.7	6.6	33.1	61.2	100.0	100.0	17.2	37.4	7.6	6.5
0.01	0.1	0.8	17.7	80.0	16.1	6.9	4.9	28.9	43.8	87.2	71.6	16.8	36.0	6.7	4.7
0.02	0.3	1.2	15.7	64.2	14.4	6.3	3.7	25.3	31.8	76.3	52.0	16.4	34.6	6.1	3.5
0.03	0.3	1.5	14.2	52.4	13.0	5.7	2.8	22.2	23.4	67.1	38.3	16.0	33.2	5.4	2.7
0.04	0.4	1.6	13.0	43.6	11.8	5.1	2.1	19.6	17.5	59.2	28.6	15.7	31.9	4.9	2.1
0.05	0.4	1.7	12.0	36.9	10.8	4.7	1.7	17.3	13.2	52.4	21.6	15.3	30.7	4.4	1.6
0.06	0.5	1.7	11.2	31.7	10.0	4.3	1.3	15.4	10.1	46.5	16.5	15.0	29.5	4.0	1.3
0.07	0.5	1.7	10.5	27.5	9.2	3.9	1.1	13.7	7.8	41.5	12.8	14.6	28.5	3.7	1.0
0.08	0.5	1.7	9.9	24.2	8.6	3.6	0.9	12.3	6.1	37.1	10.0	14.3	27.4	3.4	0.8
0.09	0.6	1.7	9.4	21.4	8.1	3.3	0.7	11.0	4.8	33.3	7.9	14.0	26.5	3.1	0.7
0.10	0.6	1.7	9.0	19.2	7.7	3.0	0.6	9.9	3.8	29.9	6.3	13.7	25.6	2.8	0.5
0.11	0.6	1.6	8.6	17.3	7.3	2.8	0.5	8.9	3.1	27.0	5.0	13.4	24.8	2.6	0.4
0.12	0.6	1.6	8.3	15.8	6.9	2.6	0.4	8.1	2.5	24.4	4.1	13.2	24.0	2.4	0.4
0.13	0.6	1.6	8.0	14.4	6.6	2.4	0.3	7.3	2.0	22.2	3.3	12.9	23.3	2.2	0.3
0.14	0.7	1.5	7.7	13.3	6.3	2.2	0.3	6.7	1.7	20.2	2.7	12.7	22.6	2.1	0.3
0.15	0.7	1.5	7.5	12.3	6.1	2.1	0.2	6.1	1.4	18.4	2.3	12.4	21.9	1.9	0.2
0.16	0.7	1.5	7.2	11.4	5.9	2.0	0.2	5.6	1.2	16.8	1.9	12.2	21.3	1.8	0.2
0.17	0.7	1.4	7.0	10.7	5.7	1.8	0.2	5.1	1.0	15.4	1.6	12.0	20.7	1.6	0.2
0.18	0.7	1.4	6.9	10.0	5.5	1.7	0.2	4.7	0.8	14.1	1.3	11.8	20.1	1.5	0.1
0.19	0.7	1.4	6.7	9.4	5.3	1.6	0.1	4.3	0.7	13.0	1.1	11.6	19.6	1.4	0.1
0.20	0.7	1.4	6.5	8.9	5.2	1.5	0.1	4.0	0.6	12.0	1.0	11.4	19.1	1.3	0.1
0.21	0.7	1.3	6.4	8.4	5.0	1.4	0.1	3.7	0.5	11.0	0.8	11.2	18.6	1.3	0.1
0.22	0.7	1.3	6.3	8.0	4.9	1.3	0.1	3.4	0.4	10.2	0.7	11.0	18.2	1.2	0.1
0.23	0.7	1.3	6.1	7.6	4.8	1.3	0.1	3.1	0.4	9.5	0.6	10.8	17.7	1.1	0.1
0.24	0.7	1.3	6.0	7.3	4.7	1.2	0.1	2.9	0.3	8.8	0.5	10.7	17.3	1.0	0.1
0.25	0.7	1.2	5.9	7.0	4.6	1.1	0.1	2.7	0.3	8.2	0.5	10.5	16.9	1.0	0.1
0.26	0.7	1.2	5.8	6.7	4.5	1.1	0.1	2.5	0.2	7.6	0.4	10.4	16.5	0.9	0.0
0.27	0.8	1.2	5.7	6.4	4.4	1.0	0.1	2.3	0.2	7.1	0.4	10.2	16.2	0.9	0.0
0.28	0.8	1.2	5.6	6.2	4.3	1.0	0.0	2.2	0.2	6.6	0.3	10.1	15.8	0.8	0.0
0.29	0.8	1.2	5.6	6.0	4.2	0.9	0.0	2.1	0.2	6.2	0.3	9.9	15.5	0.8	0.0
0.30	0.8	1.2	5.5	5.7	4.1	0.9	0.0	1.9	0.1	5.8	0.2	9.8	15.2	0.7	0.0
0.31	0.8	1.1	5.4	5.6	4.1	0.8	0.0	1.8	0.1	5.4	0.2	9.7	14.9	0.7	0.0
0.32	0.8	1.1	5.3	5.4	4.0	0.8	0.0	1.7	0.1	5.1	0.2	9.5	14.6	0.7	0.0
0.33	0.8	1.1	5.3	5.2	3.9	0.8	0.0	1.6	0.1	4.8	0.2	9.4	14.4	0.6	0.0
0.34	0.8	1.1	5.2	5.1	3.9	0.7	0.0	1.5	0.1	4.5	0.2	9.3	14.1	0.6	0.0
0.35	0.8	1.1	5.1	4.9	3.8	0.7	0.0	1.4	0.1	4.3	0.1	9.2	13.9	0.6	0.0
0.36	0.8	1.1	5.1	4.8	3.8	0.7	0.0	1.3	0.1	4.0	0.1	9.1	13.6	0.6	0.0
0.37	0.8	1.1	5.0	4.7	3.7	0.6	0.0	1.3	0.1	3.8	0.1	9.0	13.4	0.5	0.0
0.38	0.8	1.1	5.0	4.6	3.7	0.6	0.0	1.2	0.1	3.6	0.1	8.9	13.2	0.5	0.0
0.39	0.8	1.0	4.9	4.4	3.6	0.6	0.0	1.1	0.1	3.4	0.1	8.7	13.0	0.5	0.0
0.40	0.8	1.0	4.9	4.3	3.6	0.6	0.0	1.1	0.1	3.2	0.1	8.7	12.8	0.5	0.0

M. Cook, 2007. Yellowedge grouper from the Gulf of Mexico, USM dissertation.

APPENDIX D

RESULTS OF YIELD-PER-RECRUIT (YPR) AND SPAWNING STOCK BIOMASS-PER-RECRUIT (SSB/R) WHEN NATURAL MORTALITY (*M*) EQUALS 0.090

F represents fishing mortality, *n* is numbers, F is females and M is males.

F	Catch *n*	YPR (kg)	Stock *n*	Stock weight (kg)	Mean age (years)	Spawning stock numbers		SSB/R (kg)		Spawning potential (%)		Mean generation time (years)		Expected spawnings	
						F	M	F	M	F	M	F	M	F	M
0.00	0.0	0.0	11.6	37.6	11.4	4.5	1.8	17.0	14.7	100.0	100.0	15.6	31.7	4.3	1.7
0.01	0.1	0.3	10.7	31.9	10.4	4.1	1.4	15.1	11.1	88.6	75.8	15.2	30.4	3.9	1.4
0.02	0.1	0.5	10.0	27.4	9.6	3.7	1.1	13.4	8.5	78.8	58.1	14.9	29.3	3.5	1.1
0.03	0.2	0.6	9.4	23.9	8.9	3.4	0.9	11.9	6.6	70.3	45.0	14.6	28.2	3.2	0.8
0.04	0.2	0.7	8.9	21.1	8.4	3.1	0.7	10.7	5.2	62.9	35.3	14.2	27.2	2.9	0.7
0.05	0.3	0.8	8.5	18.7	7.8	2.9	0.6	9.6	4.1	56.4	27.9	13.9	26.3	2.7	0.6
0.06	0.3	0.9	8.1	16.8	7.4	2.7	0.5	8.6	3.3	50.8	22.3	13.7	25.4	2.5	0.5
0.07	0.3	0.9	7.8	15.2	7.0	2.5	0.4	7.8	2.6	45.9	17.9	13.4	24.6	2.3	0.4
0.08	0.4	0.9	7.5	13.9	6.7	2.3	0.3	7.1	2.1	41.5	14.5	13.1	23.8	2.1	0.3
0.09	0.4	0.9	7.3	12.7	6.4	2.1	0.3	6.4	1.7	37.7	11.9	12.9	23.1	1.9	0.3
0.10	0.4	1.0	7.0	11.7	6.1	2.0	0.2	5.8	1.4	34.3	9.8	12.6	22.4	1.8	0.2
0.11	0.4	1.0	6.8	10.9	5.9	1.8	0.2	5.3	1.2	31.3	8.1	12.4	21.8	1.7	0.2
0.12	0.5	1.0	6.6	10.1	5.7	1.7	0.2	4.9	1.0	28.6	6.8	12.1	21.2	1.6	0.2
0.13	0.5	1.0	6.4	9.5	5.5	1.6	0.2	4.5	0.8	26.2	5.7	11.9	20.6	1.4	0.1
0.14	0.5	1.0	6.3	8.9	5.3	1.5	0.1	4.1	0.7	24.1	4.8	11.7	20.0	1.3	0.1
0.15	0.5	1.0	6.1	8.4	5.2	1.4	0.1	3.8	0.6	22.1	4.1	11.5	19.5	1.3	0.1
0.16	0.5	1.0	6.0	7.9	5.0	1.3	0.1	3.5	0.5	20.4	3.5	11.3	19.0	1.2	0.1
0.17	0.5	0.9	5.9	7.5	4.9	1.3	0.1	3.2	0.4	18.9	3.0	11.1	18.5	1.1	0.1
0.18	0.5	0.9	5.8	7.2	4.8	1.2	0.1	3.0	0.4	17.4	2.5	11.0	18.1	1.0	0.1
0.19	0.5	0.9	5.7	6.8	4.6	1.1	0.1	2.7	0.3	16.2	2.2	10.8	17.7	1.0	0.1
0.20	0.5	0.9	5.6	6.5	4.5	1.1	0.1	2.6	0.3	15.0	1.9	10.6	17.2	0.9	0.1
0.21	0.6	0.9	5.5	6.2	4.4	1.0	0.1	2.4	0.2	14.0	1.7	10.5	16.8	0.9	0.0
0.22	0.6	0.9	5.4	6.0	4.3	0.9	0.1	2.2	0.2	13.0	1.5	10.3	16.5	0.8	0.0
0.23	0.6	0.9	5.3	5.8	4.3	0.9	0.0	2.1	0.2	12.1	1.3	10.2	16.1	0.8	0.0
0.24	0.6	0.9	5.2	5.6	4.2	0.9	0.0	1.9	0.2	11.3	1.1	10.0	15.8	0.7	0.0
0.25	0.6	0.9	5.2	5.4	4.1	0.8	0.0	1.8	0.1	10.6	1.0	9.9	15.5	0.7	0.0
0.26	0.6	0.9	5.1	5.2	4.0	0.8	0.0	1.7	0.1	9.9	0.9	9.7	15.1	0.7	0.0
0.27	0.6	0.9	5.0	5.0	4.0	0.7	0.0	1.6	0.1	9.3	0.8	9.6	14.9	0.6	0.0
0.28	0.6	0.9	5.0	4.9	3.9	0.7	0.0	1.5	0.1	8.7	0.7	9.5	14.6	0.6	0.0
0.29	0.6	0.9	4.9	4.7	3.8	0.7	0.0	1.4	0.1	8.2	0.6	9.4	14.3	0.6	0.0
0.30	0.6	0.9	4.8	4.6	3.8	0.6	0.0	1.3	0.1	7.7	0.6	9.3	14.1	0.5	0.0
0.31	0.6	0.9	4.8	4.5	3.7	0.6	0.0	1.2	0.1	7.3	0.5	9.1	13.8	0.5	0.0
0.32	0.6	0.8	4.7	4.3	3.7	0.6	0.0	1.2	0.1	6.9	0.5	9.0	13.6	0.5	0.0
0.33	0.6	0.8	4.7	4.2	3.6	0.6	0.0	1.1	0.1	6.5	0.4	8.9	13.4	0.5	0.0
0.34	0.6	0.8	4.6	4.1	3.6	0.5	0.0	1.0	0.1	6.2	0.4	8.8	13.2	0.4	0.0
0.35	0.6	0.8	4.6	4.0	3.5	0.5	0.0	1.0	0.0	5.8	0.3	8.7	13.0	0.4	0.0
0.36	0.6	0.8	4.6	3.9	3.5	0.5	0.0	0.9	0.0	5.5	0.3	8.6	12.8	0.4	0.0
0.37	0.6	0.8	4.5	3.9	3.5	0.5	0.0	0.9	0.0	5.2	0.3	8.5	12.6	0.4	0.0
0.38	0.6	0.8	4.5	3.8	3.4	0.5	0.0	0.8	0.0	5.0	0.3	8.4	12.4	0.4	0.0
0.39	0.6	0.8	4.4	3.7	3.4	0.4	0.0	0.8	0.0	4.7	0.2	8.3	12.3	0.4	0.0
0.40	0.7	0.8	4.4	3.6	3.4	0.4	0.0	0.8	0.0	4.5	0.2	8.2	12.1	0.3	0.0

APPENDIX E

SUMMARY OF YELLOWEDGE GROUPER LIFE HISTORY PARAMETERS AND BIOLOGICAL REFERENCE POINTS

von Bertalanffy growth equation parameters:

L_∞ = 970.8 mm
K = 0.063
t_0 = -4.84
W_∞ = 12.95 kg

Maximum ages observed: males 85 years, females 33 years

Maximum total length (TL) observed: 1,170 mm

Maximum weight observed: 17.8 kg whole weight

Females begin to sexually mature at 475 mm TL, age 2 years

Length and age at 50% sexual maturity:
All data: 512 mm TL, age 7 years
Eastern Gulf of Mexico: 511 mm TL, 7 years
Western Gulf of Mexico: 533 mm TL, 7 years

All females sexually mature by 640 mm TL, age 17 years

Length and age at 50% sexual transition:
All data: 840 mm TL, age 23 years
Eastern Gulf of Mexico: 811 mm TL, 22 years
Western Gulf of Mexico: 865 mm TL, 25 years

Depth range: 35-390 m (mean=125 m)

Natural mortality: M=0.048

F_{Max} = 0.067

$F_{0.1}$ = 0.041

$F_{30\%\ SPR}$ Females = 0.100 $F_{30\%\ SPR}$ Males = 0.038

$F_{50\%\ SPR}$ Females = 0.054 $F_{50\%\ SPR}$ Males = 0.026

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