12-31-2010

Aspects of the Life History of the Snowy Grouper, Epinephelus niveatus, in the Gulf of Mexico

Kelley Kowal
University of South Florida

Aspects of the Life History of the Snowy Grouper, *Epinephelus niveatus*,

in the Gulf of Mexico

by

Kelley D. Kowal

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science
College of Marine Science
University of South Florida

Major Professor: Kendra Daly, Ph.D.
Richard Cody, Ph.D.
Robert Muller, Ph.D.
Ernst Peebles, Ph.D.

Date of Approval:
October 26, 2010

Keywords: age, growth, reproduction, hermaphroditic, overfishing

Acknowledgements

I would like to thank my committee members Drs. Kendra Daly, Richard Cody, Robert Muller, and Ernst Peebles for all of their assistance, patience, time, and inspiration. Thanks to Janet Tunnell, Linda Lombardi-Carlson, Gary Fitzhugh, Jennifer Potts, Dr. Harry Grier, Christy Stephenson, and Noretta Perry for all of the time, energy, support, and information that you all provided; I could not have done this without all of you. I am grateful to Chris Skeie, Katie Toth, David Crewz, and Patti Busby for all the positive energy and motivation they provided over the years it has taken me to complete this work. I would also like to thank my parents David and Sharon Kowal for instilling in me the value of education and providing me with the strength and perseverance to follow through with what I started. Finally, special thanks to Katie and Julia Reeves. You have both helped me in so many ways and have provided me with support and kindness through some of the toughest days.

Table of Contents

List of Tables

List of Figures

Abstract

Knowledge of many life history parameters are essential to properly assess and manage fish species. Although the snowy grouper, *Epinephelus niveatus,* is a commercially valuable fish, which is harvested throughout the Gulf of Mexico, little is known about its age, growth, and reproduction from this region. In this study, snowy grouper from the northern and eastern regions of the Gulf of Mexico were examined primarily using commercially-derived samples that were collected between 1984 and 2004. A total of 1,200 snowy grouper with fork lengths between 242 and 1,190 mm were collected. Sectioned saggital otoliths were used to determine the age of 774 specimens which varied from 1 to 44 years, considerably older than previously recorded. Ninety gonad samples were histologically examined; the sample population consisted of 82 females in various stages of development, 3 males, and 5 transitional fish. Female fish had ages that ranged between 3 to 14 years, with fork lengths from 330 to 880 mm and male fish had ages that were between 17 to 25 years in age, with fork lengths from 955 to 1,080 mm. Transitional fish had ages from 6 to 13 years, with fork lengths from 474 to 930 mm. The results of this study suggest that sexual maturity in females was reached around five or six years, and transition in some fish occurred as early as five years but was observed in older fish. The few males that were collected were older and larger than those fish identified as

females. Snowy grouper grow slowly but consistently throughout the first 15 years or up to approximately 1,000 mm in length, at which point growth slows. The von Bertalanffy growth model fitted to all the observed data was $L_{(t)} = 1{,}057 * (1-e^{-0.0939(t+2.5375)})$. Snowy grouper recruit into the fishery at around age two, approximately 300 mm fork length. The truncated nature of the age distribution and low number of males collected suggest that snowy grouper in the Gulf of Mexico are likely experiencing overfishing, and more research on the species is necessary to facilitate proper management and conservation.

Introduction

The snowy grouper, *Epinephelus niveatus*, is a commercially valuable deepwater serranid, with a distribution ranging from North Carolina to Brazil (Smith, 1971; Huntsman and Dixon 1976; Matheson and Huntsman, 1984; Moore and Labisky, 1984), including the Caribbean and the Gulf of Mexico (Bohlke and Chaplin, 1968). Until recently this species was thought to exist in the eastern Pacific (Knaggs et al., 1975; Miller and Lea, 1976; Fitch and Schultz, 1978; Matheson and Huntsman, 1984, Moore and Labisky, 1984), however, the Pacific fish has been identified as a separate species, *Epinephelus niphobles* (Carpenter, 2002). Recent genetics advancements have resulted in the reclassification of groupers of the genera *Mycteroperca* and *Epinephelus* (Family: Serrenidae). The snowy grouper are now classified as part of the genus *Hyporthodus* (Craig and Hastings, 2007). The new genus name has yet to be adopted by the Integrated Taxonomic Information System, which provides reliable, coordinated taxonomic information; therefore, snowy grouper in this study will be referred to as *Epinephelus niveatus*.

The annual commercial harvest of snowy grouper off the Gulf coast of Florida varied from 112,341 lbs in 1996 to 191,263 lbs in 2009, with a maximum of 220,926 lbs in 2006 (FWRI web, 10/5/10). The annual catch, however, may not be accurate due to the misidentification of the species by commercial fishers. For example, 76,617 lbs of grouper were listed as “other” in 2009. In addition, federal commercial grouper fishing regulations in the Gulf of Mexico were modified in 2001, with the implementation of a seasonal closure on the harvest and prohibited sales of shallow-water grouper (red, gag, and black). In 2004, in addition to the seasonal closure of shallow-water groupers, the commercial quota (in millions of pounds) was lowered for both shallow- and deep-water groupers. Hence, changes in fishing regulations of other grouper may have resulted in an increased harvest of snowy grouper in Gulf waters during the first half of this decade as fishers moved to deeper waters to avoid catching shallow-water groupers.

Adult *E. niveatus* can be difficult to distinguish from other grouper species (Figure 1). Identifying characteristics include 11 spines and 13 to 15 soft rays on the dorsal fin, and an anal fin with 3 spines and 15 soft rays. Adults have a dark brown coloration and black along the margin of the dorsal spines. Juveniles are dark brown with white spots in vertical rows on the sides of the body, often extending onto the head and dorsal fin (Carpenter, 2002). Similar to other groupers, *E. niveatus* has a black saddle on the caudal peduncle (Smith, 1971).

Information on the ecology, habitat, and diet of the snowy grouper is limited to studies from North Carolina and the Florida Keys. Larval snowy grouper were described and observed in the Florida Straits at the surface and 300 feet (91 m) (Presley, 1970; Figure 2). Juveniles and young adults were found at depths as shallow as 35 m (Moore and Labisky, 1984; Matheson and Huntsman, 1984). Adult snowy grouper are commonly found on the upper continental slope at depths greater than 60 meters and have been reported to depths of 200 m or more (Huntsman and Dixon, 1976; Roe, 1976; Low and Ulrich, 1983). In a recent study of the species (Wyanski et al., 2000), snowy grouper were found between 46 m and 256 m off the Carolinas. They noted that larger fish (> 600 mm) were most often found at depths greater than 100 m, while smaller fish (< 600 mm) were generally found at depths less than 100 m. Thus, snowy grouper appear to migrate to deeper water as older adults.

In the western Atlantic, snowy grouper are found on the upper continental shelf consisting of rocky ledges, cliffs, and ridges of eroded limestone, with vertical relief up to 10 m and fast currents (Matheson and Huntsman, 1984; Parker and Mays, 1998; Wyanski et al., 2000). Bielsa and Labisky (1987) investigated the diet of snowy grouper and found that bony fishes (osteichthyes) were the dominant prey item, contributing up to 78% of the total diet. Cephalopods and brachyuran crabs were also consumed. Osteichthyes were consumed similarly among the three size-classes of snowy grouper assessed (< 500 mm, 511 – 685 mm, and > 686 mm TL). Cephalopods composed a larger portion of the prey items in the intermediate and large sized grouper although they were also consumed by smaller fish. The small size class numerically favored gastropods, while brachyuran crabs were preferred by the largest fish. The intermediate-size grouper had the most diverse diet in terms of the number of species.

Snowy grouper are a relatively large, long-lived species and can develop a robust body. The maximum oldest age reported for snowy grouper was 29 years old with lengths of 1,137 mm TL (Wyanski et al., 2000), and with a body weight up to 29 kg (Moore and Labisky, 1984; Parker and Mays, 1998). Estimated growth rates for snowy grouper vary depending on the location. A population from the Florida Keys had a growth coefficient (k) of 0.087 (Moore and Labisky, 1984), while another study in the Carolinas reported a k value of 0.063 (Matheson and Huntsman, 1984), suggesting that snowy grouper in Florida may have a faster growth rate.

Snowy grouper are protogynous hermaphrodites, i.e., where some functional females transition to functional males (Matheson and Huntsman, 1984; Moore and Labisky, 1984; Carpenter, 2002). It is not known if all females eventual transition to males. Sexual maturity is reached by females at ages 3 – 5 years (450 – 500 mm TL) but the first males were not observed until age six. Individuals in spawning condition were evident from April through July in the Florida Keys (Moore and Labisky, 1984; Parker and Mays, 1998) and April through September in North and South Carolina (Wyanski et al., 2000). Even though there is little information available on the early life history of the snowy grouper, it is known that the eggs and larvae are pelagic (Presley, 1970; Moore and Labisky; 1984; Parker and Mays, 1998). Snowy grouper larvae have been reported only in the Florida Keys during the 1960s at two depths, the surface

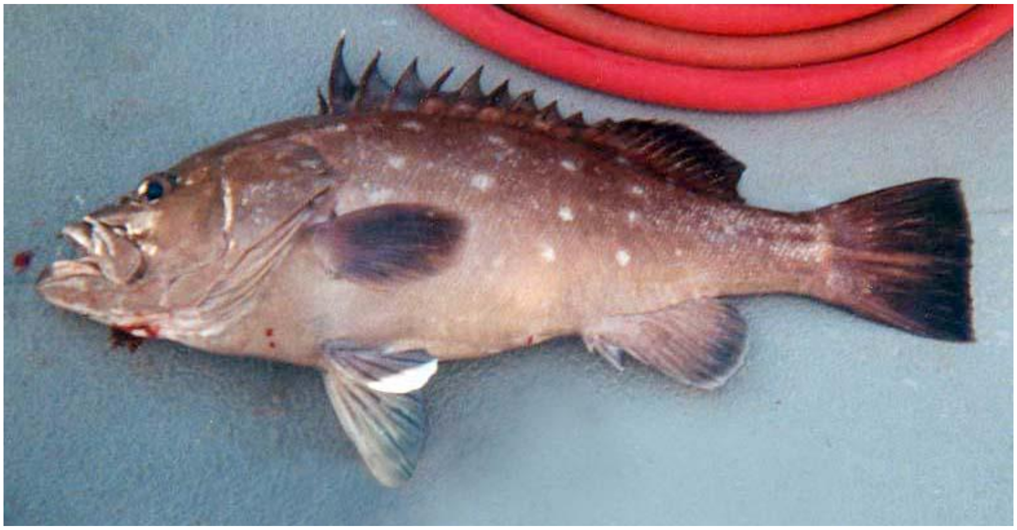


Figure 1. *Epinephelus niveatus*, note identifying white spots, found on juveniles and young adults.

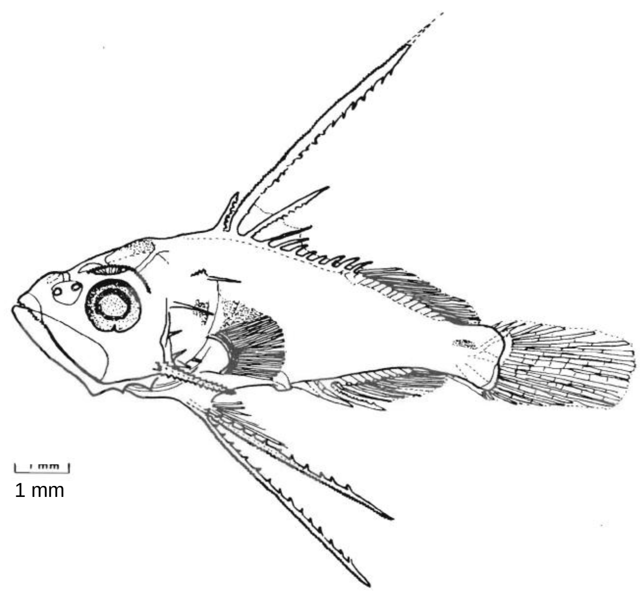


Figure 2. *E. niveatus* larva 10.04 mm TL, collected from the Florida Straits (after Presley, 1970).

and 91 m (Presley, 1970). Based on other studies of grouper species, eggs should hatch in 1 – 5 days depending on environmental conditions (Tucker, 1994; Jagadis et al., 2006).

The Deepwater Horizon oil spill which occurred between April 20 and July 15, 2010, in the northern Gulf of Mexico highlights the urgent need for baseline parameters on population abundances, biology, ecology, and life histories of fish species in the Gulf. With little known of the ecological consequences resulting from an oil spill at a depth of 1,259 m (RIGZONE 2010), and the use of chemical dispersants of unknown composition, the Deepwater Horizon event has the potential to be a massive long-term ecological disaster. The ultimate extent of the disaster may not be evident for many years, as the oil and the chemical dispersants interact with the ecosystem and food-web. Areas of snowy grouper habitat in the northern portion of the Gulf are located in the region damaged by the spill. Long-lived, deepwater species, such as snowy grouper, could experience long-term biological effects. Without information on the interannual variability (Lehodey et al., 2006) and ecology of Gulf of Mexico marine species, it will be difficult to assess the long-term effects of the spill.

Despite snowy grouper being commercially exploited, with variable annual harvests, throughout the Gulf of Mexico, little is known about the life history or growth rates of populations in this region. The goal of this study was to determine the vital life history parameters of the snowy grouper collected between 1984 and 2004, which included (1) age structure, growth, and age–

length relationships, (2) relationships between fish length, age, and otolith weight and (3) size and age at maturity, at sex change, and reproductive seasonality of snowy grouper in the Gulf of Mexico. This life history information is critical to managing the fisheries and for evaluating the effects of natural and human-induced stressors, such as oil spills, hurricanes, and climate warming.

Materials and Methods

Specimen Acquisition

The data and samples for this study were provided by the National Marine Fisheries Service (NMFS) Laboratory at Panama City, Florida. The bulk of the samples, otoliths, and gonads, were obtained from commercial fish houses through the NMFS Trip Interview Program (TIP). TIP port samplers collect length and weight data from commercially harvested fish, and also they may collect data regarding the area fished, gear-type and quantity of gear used, and time fished (Molina, 1999). In addition TIP samplers may collect hard parts and tissue samples for later analyses. The data collected in the Gulf of Mexico as part of the TIP were cataloged and archived at the NMFS Panama City Laboratory. Other samples were collected via the NMFS Bottom Longline Survey and the Gulf States Marine Fisheries Commission (GSMFC) Fishery Information Network (FIN) biological sampling program. The 1,169 commercially–derived snowy grouper samples were collected between 1984 and 2004. The majority of samples were landed in Florida (83%) and the remaining samples were landed in Louisiana (12.1%), Mississippi (1.33%), and Texas (0.01%). Thirty-four (3%) of the samples were landed in the Gulf; although the landing state or area fished was not recorded. The specific area fished was unavailable for the majority of samples because it is not required and considered proprietary by the commercial fishers. Scientific collections by the NMFS Bottom Longline Survey accounted for 2% of the total, and recreational anglers contributed 0.5% of the samples. Specimens collected from the NMFS project included the area fished, depth, sea surface–temperature, and temperature–oxygen profiles from the surface to depth. Figure 3 shows the known capture locations of some of the snowy grouper used in this study.

Only two types of gear, longline and hook–and–line, were used successfully to harvest this species during the time period studied. Longline gear was used to harvest the majority of the fish, accounted for 826 samples collected through the TIP and all of the fish collected by the NMFS project. Hook–and–line harvest accounted for 174 samples, and included all of the samples collected from recreationally harvested fish (RecFIN) and a small portion of the commercial samples. The remaining 200 samples were reported as unspecified gear.

Fork and/or total lengths (mm) were recorded for all samples. Fork length is measured from the anterior-most tip of the fish (mouth closed) to the center of the fork in the tail (Nickum et al., 2004.). Total length is measured from the posterior-most tip of the nose, also with the mouth closed, to the tip of the longest lobe of the caudal fin. A linear regression model for fork length was used to compare the two types of measurements. The resulting model (Eq. 1) was used to acquire missing fork lengths;

$$FL = 0.9684 * TL + 5.3578 \qquad \text{(Eq. 1)},$$

where *FL* is fork length (mm) and *TL* is total length (mm) ($N = 144$, $P < 0.001$, $R^2 = 0.99$; Figure 4). All reported lengths are in fork length. Weights were available for 614 fish. Gutted (organs of abdomen removed) weights were generally reported in kilograms, with ten fish the gutted weights were reported in pounds and ounces in 1993. Total weight (kg) was provided for 31 specimens. No data were available for whole and gutted weights from the same fish. No weights were provided for the remaining 586 samples.

Age and Growth

Approximately 1,000 left otoliths were used for age determinations. If the left otolith was lost, broken, damaged, or not collected, the right otolith was used (~ 58). Whole otoliths were weighed to the nearest milligram prior to sectioning (Figure 5). Large otoliths were attached to cutting paper using hot glue. The core was marked as a guide for sectioning, by drawing a transverse pencil line anterior to the core and posterior to the junction of the ostium and sulcus. Smaller, more delicate otoliths were placed in molds, embedded in Araldite® resin, and cured at 60˚C for 24 hours. After curing, the core was marked and the embedded otoliths were attached to cutting paper with hot glue. All otoliths were

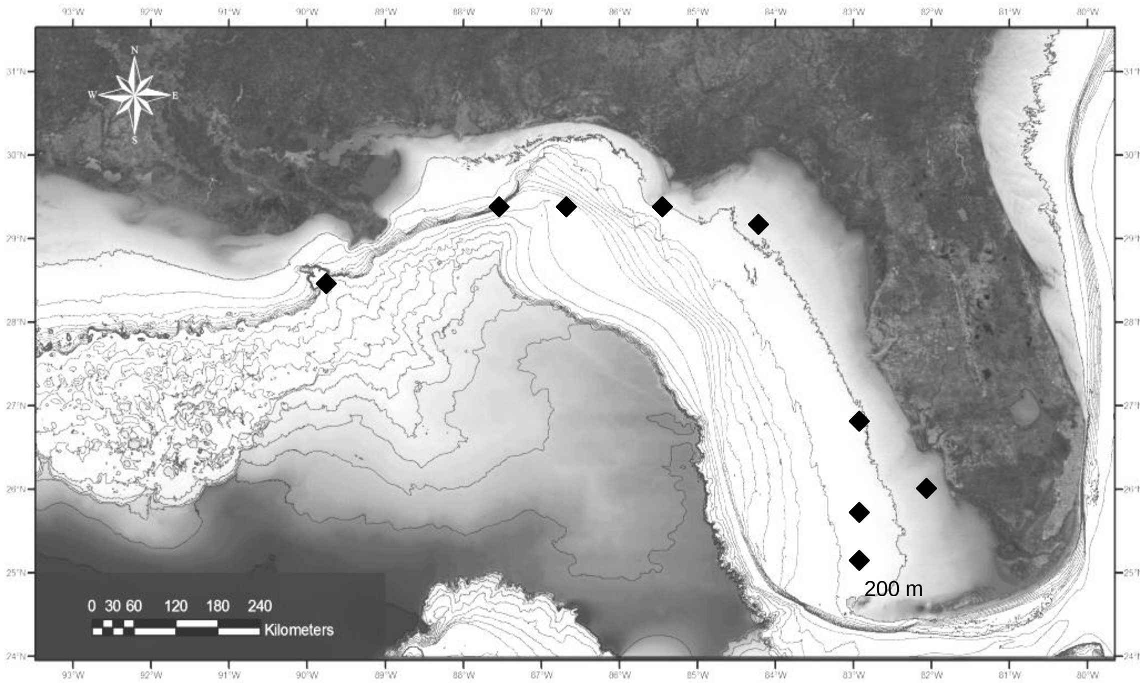


Figure 3. A map of the Gulf of Mexico, showing the locations where *E. niveatus* were collected 1993, 2003, and 2004. Samples noted above were acquired primarily from the scientific sampling. Other locations are not known. (Basemap courtesy of Lisa Robbins, USGS.)

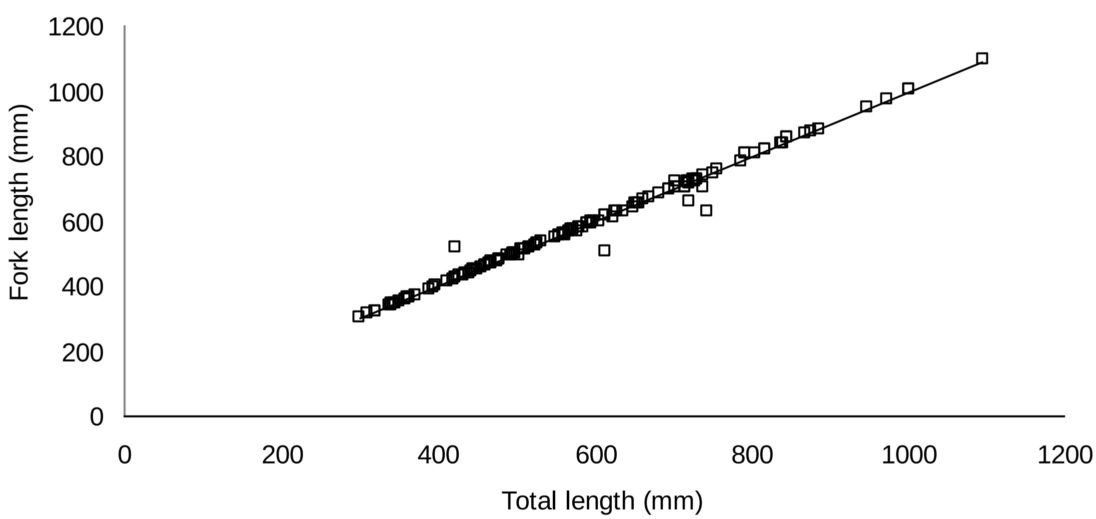
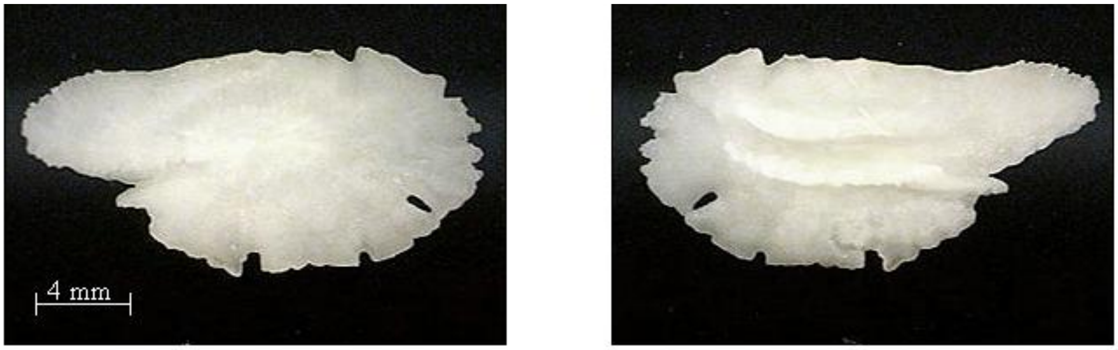


Figure 4. Relationship between total length (*TL*) and fork length (*FL*) for *E. niveatus* collected in the Gulf of Mexico. The following regression model was created: $FL = 0.9864 * TL + 5.3578$, N = 144, P < 0.0001, $R^2 = 0.99$.

A



B

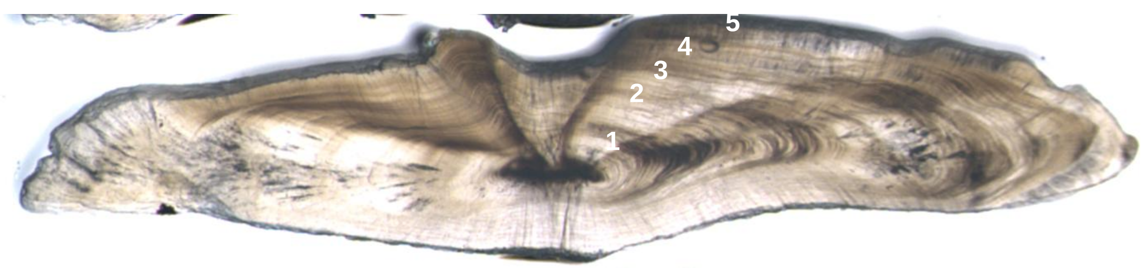


Figure 5. Examples of *E. niveatus* otoliths. (A) Whole otolith, and (B) a sectioned otolith from an age five fish with a fork length of 495 mm analyzed during this study. Otoliths in (A) from www.sefscpanamalab.noaa.gov/content/40_Fisheries_Biology/10_Otolith_Guide/Fusiform/Snowy Grouper.jpg.

cut near the core into three transverse sections, 0.5 mm thick, using a multi-blade Isomet® low–speed saw. Otolith sections were then rinsed, completely dried on blotter paper, and mounted onto slides using Flo-Texx® mounting medium. The sections were viewed through a dissecting microscope using transmitted or reflected light and the annuli counted. One hundred forty two of the fish were not aged because one or both otoliths were damaged, lost, or unreadable.

Fish otoliths are composed of crystalline calcium carbonate in a protein matrix that builds outward from the center through biomineralization (Pannella, 1971). The dense, opaque band associated with the slow–growth period is considered the annulus. The translucent region between the annuli is deposited during the fast–growth period. Annuli were counted on one of three axes (ventral, ventro-medial, or adjacent to the sulcus acousticus), although when following an increment, the axis sometimes changed during reading. Annuli were counted independently by two readers for all specimens. Determination of the first annulus was based on noting the size of the translucent area around the core of age–one fish. The number of annuli, the margin code, and the capture date were considered when assigning the calendar age of a fish (Jerald, 1983; VanderKooy, 2009). Ages were assigned to each fish based on a birth date of January 1. Yearly annulus formation has been observed and verified in snowy grouper from the western Atlantic (Moore and Labisky, 1984; Wyanski et al., 2000). Codes for different types of margins were assigned to samples where a margin could be discerned. In this study, the margin codes were assigned as follows: **2**, completed annulus on the margin; **4**, the translucent margin was less than 2/3 complete; and **6**, the translucent margin was greater than 2/3 complete. Specimens collected between January 1 and June 30 with a margin code of **6** were assigned an age equivalent to the annulus count plus one (Lombardi-Carlson et al., 2002). Fish captured after June 30, with partial or complete opaque zone formation were designated ages that were equal to the annulus count (Moe, 1969; Lombardi-Carlson et al., 2002).

The readers in this study were Janet Tunnell and Kelley Kowal. The initial 100 otoliths were also aged by Jennifer Potts at the NMFS Beaufort Laboratory. Ages that did not correspond between the two readers were viewed again by both readers. If the second reading did not agree within one year then the ages assigned by the individual with the most experience aging fish was used. Otoliths with an ageing disagreement of two or more years were not considered for further analysis.

Growth was evaluated by fitting the observed fork lengths and assigned ages to the von Bertalanffy growth model (Ricker, 1975) using a nonlinear regression (PROC NLIN; SAS 9.1). An ageing precision of one year or less was used for this portion of the study (Wyanski et al., 2000). Because of the absence of young–of–the–year or larval specimens in the study sample, total length of 16 larval specimens from a leaflet series published by R.F. Presley (1970) through the Florida Department of Natural Resources were included to provide information regarding the size at t_0. Those samples were collected in 1962 (N = 15) and 1963 (N = 1). Based on illustrations provided in the publication, total length was considered equivalent to fork length for the purpose of this study. The following von Bertalanffy equation (Eq. 2) was used to calculate growth:

$$L_t = L_\infty (1-e^{-k\,(t-t0)}) \qquad \text{(Eq. 2)},$$

where L_t is the length at time, t, and t is given in years, L_∞ is the maximum length, k is the growth coefficient (the rate at which L_∞ is reached), and t_0 is the theoretical age at which the fish would have zero length, assuming that the fish always grew according to the pattern described by the model (Ricker, 1975).

Age–length keys were developed to determine any perceptible trends in age structure. Observed length at capture and annual age were used to build the age–length frequencies. Age-at-length data were aggregated into 50 mm length intervals for each year and for all years combined (10 or more ages available) (Bartoo and Parker, 1983; Lombardi-Carlson et al., 2002).

The relationships between otolith weight, fork length, and age were determined. Otolith weight (mg) was recorded for 763 specimens. The left otolith was weighed unless unavailable, in which case the right otolith was weighed. Both otoliths were weighed, if available. The left and right weights were compared using linear regressions to determine whether the two sets of weights were similar, and if there was no significant difference, to generate a conversion equation, in order, to use either the right or left otolith in further analyses.

Reproduction

Gonads collected between 1993 and 2004 from 95 specimens of varying sizes were used for histological analysis. Seventy-three specimens were collected from commercial fishing vessels through the NMFS Trip Interview Program and an additional 22 specimens were collected between 1999 and 2003 during research cruises. All gonads were preserved in labeled plastic bags or jars, in 10% formalin, and archived at the NMFS Panama City Laboratory. Seventeen gonads had been previously sectioned, embedded in paraffin, and affixed to slides by the Louisiana State University Pathology Laboratory. The remaining 78 specimens were acquired from the Panama City Lab. Macroscopic analysis of 48 of the 78 specimens was performed by researchers at the Panama City Lab. Upon receipt at the Florida Fish and Wildlife Research Institute (FWRI), the specimens were transferred to labeled jars with fresh 10% formalin fixative. Prior to histological processing, the formalin was removed through a series of three rinses in water. The rinsing process was done to remove the remaining formalin from the tissues because any remaining formalin would inhibit penetration of paraffin, decreasing the success of embedding (NMFS PC Lab procedures). During the first rinse, gonads were placed in fresh water for over an hour, the water was drained, and the gonads rinsed for another hour for the second. The final rinse lasted for over 12 hours, after which the samples were transferred to embedding cassettes. Because of the poor preservation of the majority of specimens, the lobe in the best condition was used for analysis. A sample from the anterior, middle, and posterior was taken from each lobe. To remove any remaining water from the tissue samples they were dehydrated using ethanol. The samples were bathed for an hour in each of the following; 70%, 80%, and 95% ethanol, followed by a complete dehydration in 100% ethanol. The samples were then soaked twice in xylene for one minute each time. The tissue was embedded in paraffin and sections of gonad, 3 – 5 µm in thickness were cut and affixed to slides. The slides were stained with Hematoxylin and Eosin (H&E).

The reproductive condition of females and transitional fish were identified, based on characteristics modified from previous work (Wyanski et al., 2000) summarized in Table 1. Oocyte development was classified, using a more detailed set of criteria, based on work by Grier et al. (2009; Table 2).

Table 1**.** Histological criteria used to assess the reproductive stages female of *E. niveatus*

Stage	Description
Immature	Ovaries containing oogonia and previtellogenic oocytes. No evidence of prior spawning. Small gonad radius, short, less structured lamellae.
Mature regressed	Ovaries with oogonia, previtellogenic oocytes, early vitellogenic oocytes and degenerative oocytes (atretic) indicting past spawning.
Mature active	Ovaries with vitellogenic oocytes, indicative of spawning.
Recent spawn	Follicles will be empty, showing spawning has occurred.
Spent	Follicles will be empty, showing spawning has occurred.
Uncertain	Immature or regressed, with inactive ovaries, previtellogenic oocytes only. Reproductive stage is uncertain.
Transitional	Gonadal tissue with previtellogenic, vitellogenic oocytes, degenerative oocytes and forming seminiferous crypts

Modified from Hunter and Goldberg (1980); Hunter and Macewicz (1985); Wenner, 1986; Wyanski et al. (2000).

Table 2. Oocyte development in fish. Based on criteria by Grier et al. (2009)

Codes	Steps	Stages	Periods
GP	frequently form cell nests	Oogonia Proliferation	Mitosis
CNl	Leptoten	Chromatin Nucleolus (CN)	Active Meiosis I
CNz	Zygotene		
CNp	Pachytene		
CNed	Early diplotene		
PGon	One nucleolus	Primary Growth (PG)	Arrested Meiosis in late diplotene of the prophase I
PGmn	Multiple nucleoli		
PGpn	Peronucleolar		
PGod	Circumnuclear oil droplets		
PGca	Cortical alveolar		
SGe	Early secondary growth or early yolked oocytes	Secondary Growth: Vitellogenesis (yolked oocytes) (SG)	
SGl	Late secondary growth or late yolked oocytes		
SGfg	Full growth oocytes		
OMegv	Eccentric germinal vesicle (oil droplets become one globule)	Oocyte Maturation (OM)	
OMgvm	Germinal vesicle migration to animal pole (oocyte hydrates)		
OMgvb	Germinal vesicle breakdown (oocyte hydration near completion)		
OMmr	Meiosis resumes, 2^{nd} arrest (oocyte hydration complete)		Active Meiosis II & 2^{nd} arrest in metaphase II
OV	Oocyte emerges from the follicle, becomes an egg	Ovulation (OV)	

Results

Size and Weight

A total of 1,200 *E. niveatus* were collected from multiple locations in the Gulf of Mexico between 1984 and 2004. The largest percentage (83%) of fish was harvested by anglers on the west coast of Florida, from Key West to Pensacola. Ten fish were collected during the 1980s with fork lengths ranging from 242 to 737 mm, with an average of 393 mm. Fish collected during the 1990s (N = 211) had fork lengths ranging from 298 to 1,130 mm (mean = 656 mm). The largest quantity of fish was collected during the years 2000 – 2004 (N = 980), with fork lengths from 277 to 1,130 mm (mean = 629 mm). Specimens were collected during all months, with more fish collected between March and June (N = 616, 51%, Figure 6). The majority of samples (97%) were collected by commercial port samplers from commercial fishing vessels; therefore, biological and environmental data were not available (e.g., location, depth, salinity, sex). The remaining samples were collected through recreational and scientific sampling. Specimens were principally harvested using longline fishing gear (69%), with a smaller quantity harvested using hook–and–line (14%), and 16% were assigned unknown gear. For all years combined, *E. niveatus* varied in fork length from 242 mm to 1,096 mm (mean = 613 mm, N = 774) (Figure 7). Gutted weight varied from 0.1 kg to 32 kg (mean = 4.7 kg, N = 434) and whole weight ranged from 1.1 kg to 14.3 kg ($\bar{x}$ = 4.5 kg, N = 26). A plot comparing size, gutted weights, and whole weights can be found in Figure 8.

Age and Growth

Ages were assigned to 1,058 otoliths (99.3%). The initial agreement of ages between the two independent readers was 41.2%, while ages differing by 0 – 1 annulus, accounted for 73% of the aged population. There was a difference in agreement of more than one increment for 27% of the total otoliths aged, with 0.75% considered uninterpretable. Indices of error were calculated for those otoliths aged by both readers. The average percent age error was 11.4% (coefficient of variation was 1.08%) for the combined pool of specimens aged. The percentage differences in ageing agreement for all aged samples are shown in Table 3. The readability of many of the otoliths was challenging, resulting in a somewhat low initial agreement between reads. The readability of increments was difficult due to obstruction by crystalline structures, distortion of increments from clouded or opaque deformities (Figure 9), and the crowding of annuli in large otoliths. The axis of otolith growth was found to change one or more times (Figure 10) in many of the otoliths.

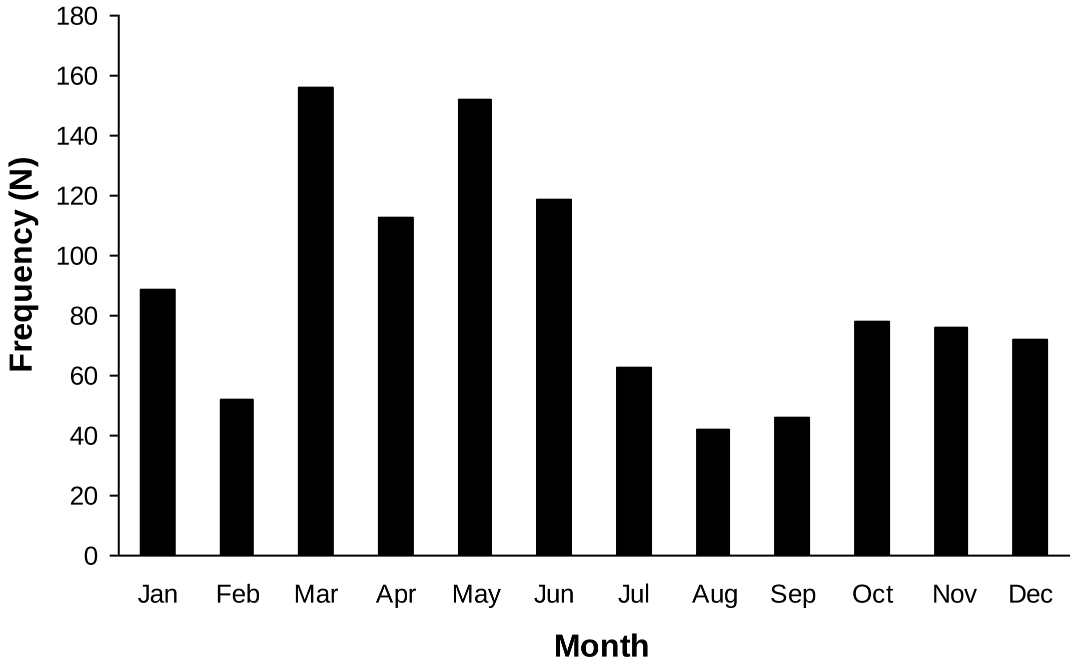


Figure 6. Number of snowy grouper collected by month between 1984 and 2004 (N = 774).

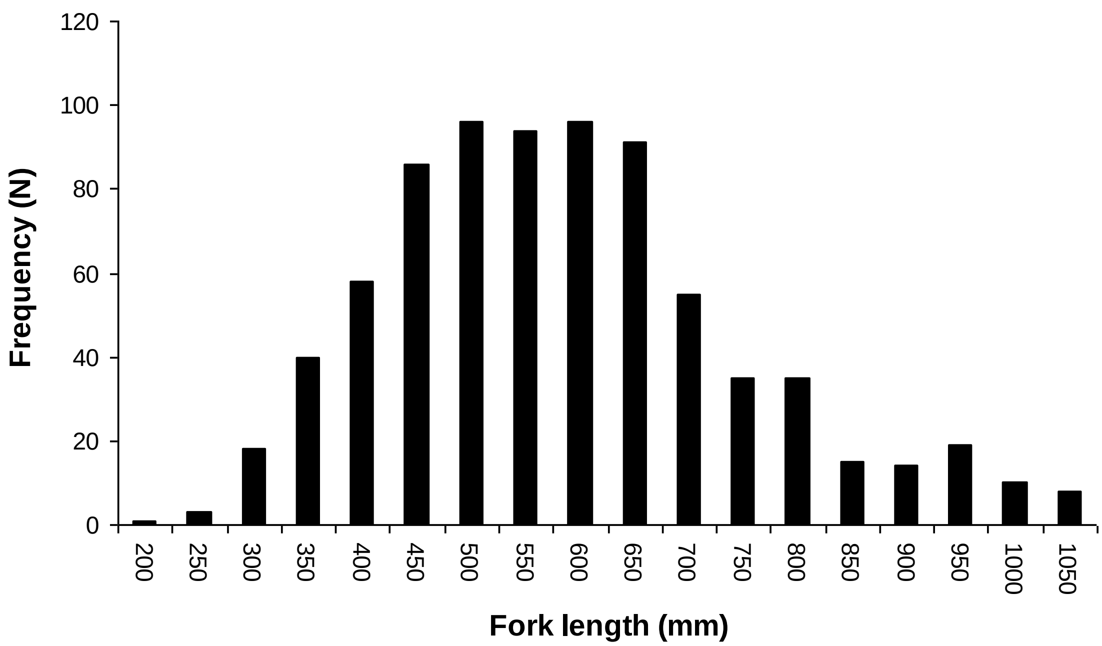


Figure 7. Length–frequency distribution of *E. niveatus* collected from the Gulf of Mexico (N = 774).

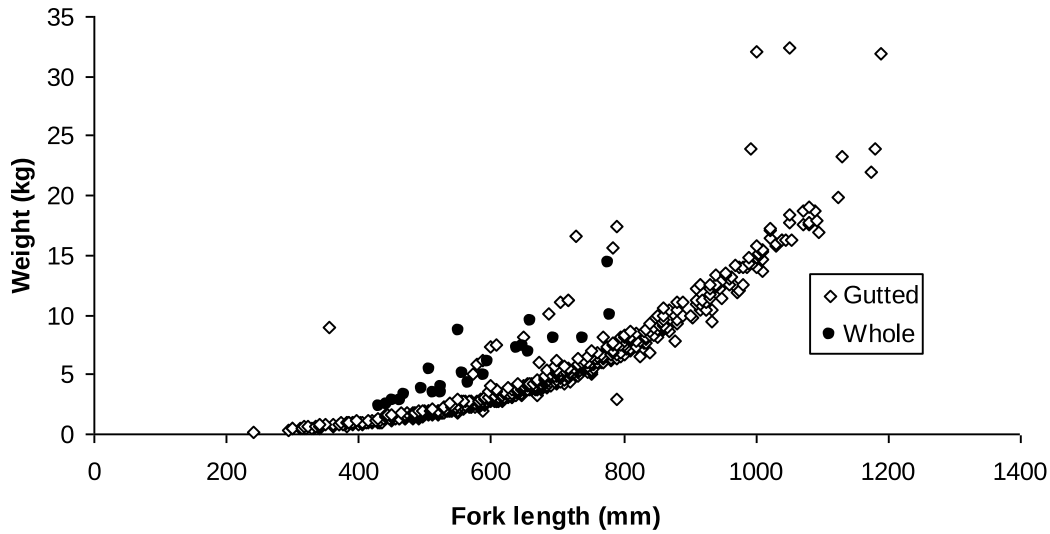


Figure 8. A comparison of fork length, gutted weights, and whole weights.

Table 3. The percentage differences between ages assigned by two readers. Zero is when both readers estimated the same number of annuli

Years discrepancy	N = 1,058	%
0	437	41.3
1	337	31.9
2	131	12.4
3	67	6.33
4	42	3.97
5	24	2.27
6	9	0.85
7	7	0.66
8	1	0.09
9	2	0.19
10	1	0.09

Epinephelus niveatus collected from the Gulf of Mexico ranged in age from 1 to 44 years (mean = 7 yrs, mode = 5 yrs), with 92% between 2 and 12 years (Figure 11). Twenty–three fish (3%) were older than age 15. The smallest specimen collected in this study was 242 mm and estimated to be two years old. The largest specimen was 1,096 mm and 31 years old. The two oldest fish were 40 and 44 years, with fork lengths of 1,070 and 1,030 mm, respectively.

Margin codes indicate the timing of opaque ring formation. The monthly frequency of margin code **2** otoliths, those with the annulus on the margin indicating recent deposition, were plotted by month to determine the spawning period of snowy grouper (Figure 12). The nature of increment formation in *E. niveatus* otoliths was unimodal indicating that one increment is deposited per year. Annulus formation in the majority of snowy grouper from the Gulf of Mexico occurred between March and June, with a peak during April and May.

The age–length keys reflect annual age structure (see Appendix). During the 1990s, snowy grouper were not well represented in commercially harvested samples, as a minimum sample size of N = 10 per year was selected for this study. Even though the number of available fish was low, an acceptable representation of ages was attained in 1991, 1992, and 1999, with the oldest fish aged at 29 years having a fork length of 972 mm. Snowy grouper from this time period entered the fishery around age two. In this study, fish under 11 years of age were most common. Fish under 16 years of age were well represented in the 2000–2004 samples and although fish between 16 and 44 years were observed, they were at a much lower frequency. Fish from this period entered the fishery between ages two and three. The oldest fish harvested was collected in 2004, at 44 years old, with a fork length of 1,030 mm.

Overall, the predominant age of fish ranged from 2 – 11 years (89%). The greatest numbers of fish were found in age classes 5 – 8, each class consisting of more than 70 fish and each individual age class ≥ 9% of the aged sample. Fish of age classes older than 15 years were rarer during the period of collection, only making up 3% of the total. During 1991, the predominant year classes were 1986 to 1989 (2 – 5 yr), 75% of the total aged that year. The number of fish sampled was low for 1992 (N = 27), with the year classes from 1984, 1986, 1988, 1989, each having three or four fish (3, 4, 6, 8 years, respectively), or 56% of the total aged. From the sampling periods 1993, 1998, and 2000, too few fish were collected to determine the year and age class predominance. Nevertheless, 1994 predominanted with seven fish aged five years (47% of the total aged). The predominant year class of 2001 was 1996 (5 yr), comprising 19% of the fish aged in that year. The next strongest year classes were 1995 and 1997, each with eight fish aged six and four, a combined for 28% of the total aged. Two year classes, 1998 and 1999, predominated in 2002, with fish ages three and four years. Combined, these two year classes accounted for 35% of the total aged from 2002.

A

B

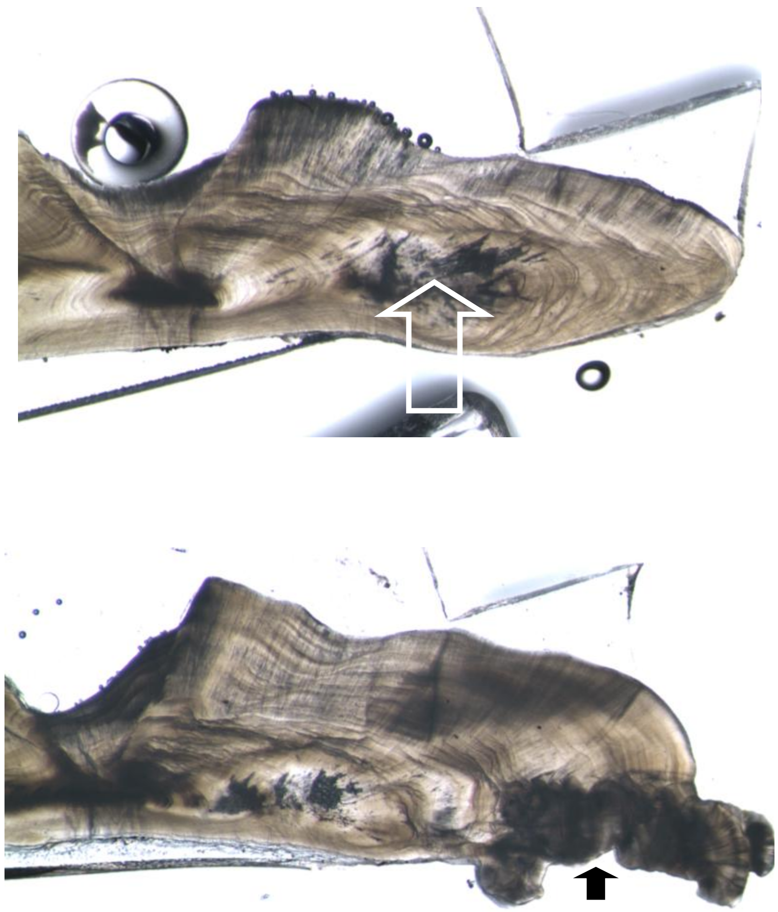


Figure 9. *E. niveatus* otoliths showing (A) crystalline structures (age 9, FL = 745 mm) and (B) opaque deformities (age 10, FL = 855 mm) which, impeded the ageing process.

Figure 10. Change in the axis of growth and crowding of annuli in large *E. niveatus* otoliths (age 34, FL = 970 mm).

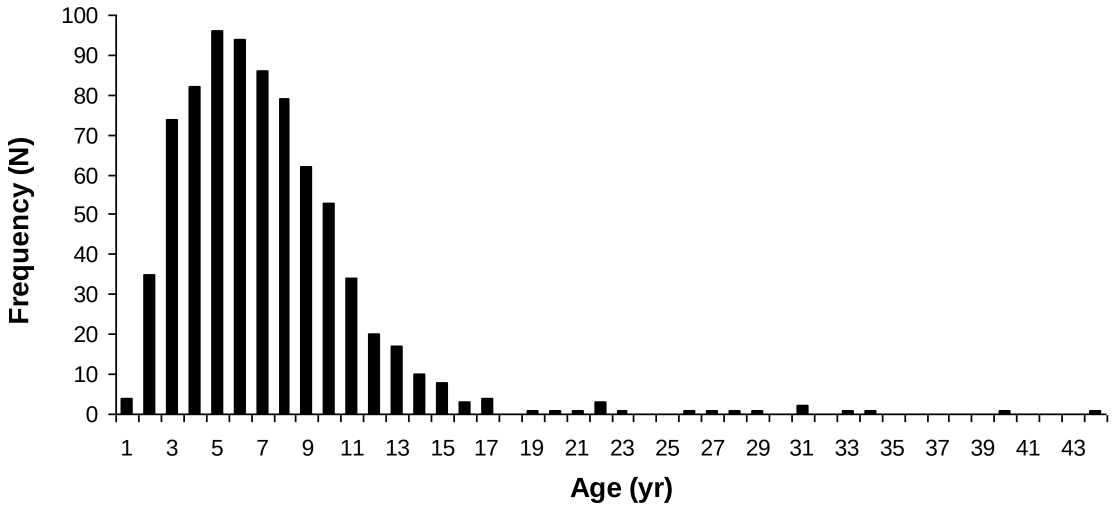


Figure 11. Age–frequency distribution of *E. niveatus* collected from the Gulf of Mexico between 1984 and 2004 (N = 774).

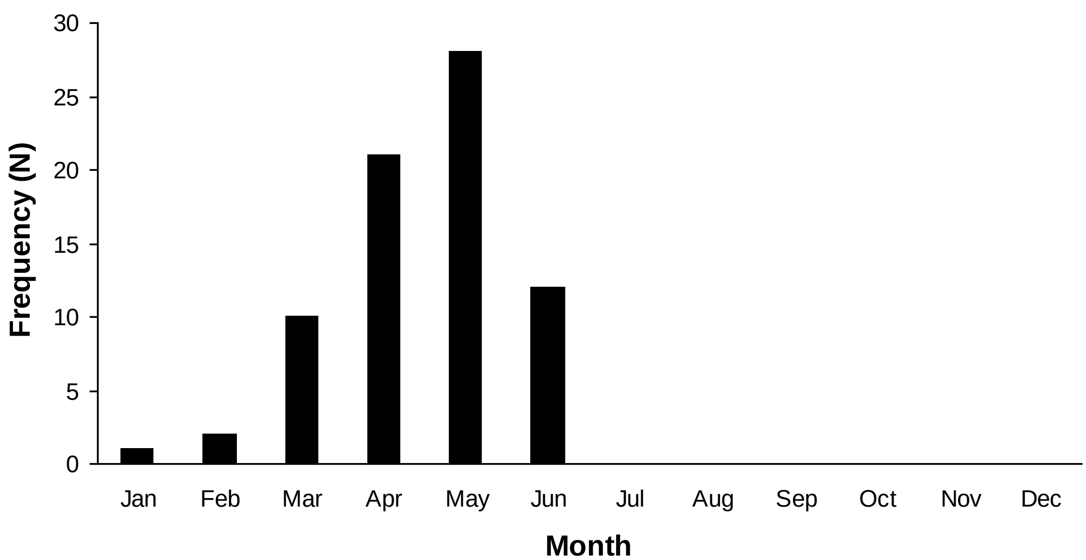


Figure 12. The frequency of occurrence of margin code **2** (completed annulus on margin) on otoliths from *E. niveatus* collected in the Gulf of Mexico between 1984 and 2004 (N = 74).

During 2003, a greater number of snowy grouper were collected than for any other previous year, accounting for 27% of all of the aged snowy grouper. Fish from 1995 and 1996 (seven and eight years), were best represented in 2003 with 27 fish each, a combined total of 26%. The year classes 1997 and 1998 (five and six yrs) were also predominant with 25 fish each, a combined total of 24%. Year classes 1999 and 1994 (four and nine yrs) were also strong, making up a combined 22%. The final year examined for this study, 2004, yielded 39% of the total aged. Year class 1997 dominated in 2004, with 44 fish aged at seven years, accounting for 15% of the annual aged total. The second predominant age class was 1996 (6 yr), with 13%. Year classes also well represented during 2004 included 2001, 2000, 1999, 1996, 1995, 1994, and 1993 (between 6 and 12% of the total aged).

The von Bertalanffy growth equation was fitted to the observed lengths and ages with combined sexes for all data (1980s, 1990s, and 2000–2004). Because known age and length data were not available for determining the size of snowy grouper at time zero (t_0), curves were fitted without $t_{0,}$ and with starting values of t_0 = 0, and t_0 = -0.25. An estimated maximum length (L_∞) of 839 mm when t_0 is omitted is extremely low, especially considering that the largest aged *E. niveatus* was 1,096 mm. The best fit to the observed length was

$$L_{(t)} = 1{,}057 * (1-e^{-0.0939(t+2.5375)}) \qquad \text{(Eq. 3).}$$

The estimated L_∞ of 1,057 mm remained the same whether starting values of t_0 = 0 or -0.25 were used in the analysis (Figure 13) and a better representation resulted than when t_0 was not included in the expression. Predicted sizes at age were similar to the observed lengths up to age 16. The departure at larger sizes is likely due to the small number of fish in the older age groups. Unfortunately, growth curves based on gender were not possible. Snowy grouper are typically gutted before landing, so that, the majority of fish could not be assigned a gender. Only one male was observed among the 69 sexed fish using the precision criterion (0 – 1 yr) for the ageing portion of this study.

The von Bertalanffy growth curves of the total sample (1980s, 1990s, 2000–2004) including the larval fish lengths from the 1960s (Presley, 1970), were determined. In addition, the growth curves of samples collected during the 1990s and the years 2000–2004 were compared using a randomization analysis (Manly, 2007). Time periods were pooled (1990 and 2000–2004) and each of these time periods run independently, which resulted in different parameter estimates than for the total sample (including the 1980s and the larval fish from the 1960s). The estimated growth parameters for the two time periods combined are L_∞ = 1,228 mm, k = 0.058, t_0 = -5.228. The estimated L_∞ in the pooled model is considerably higher than for the model using the total sample (Table 4). The growth curve for the 1990s and the 2000s were significantly different (Figure 14) from each other ($P < 0.001$) based on the results of the randomization analysis using 1,000 iterations comparing of the sum of squares of the pooled data against the sum of squares of the individual time periods.

Otolith weight (mg) was measured for 763 samples. The left otolith was measured in most cases (N = 741, 97%). If the left was unusable, the right otolith was weighed instead (N = 22, 5%), in some cases both otoliths were available, then both were weighed. The linear relationship between the weight (mg) of left and right otoliths was described by the linear regression;

$$Lt = 0.9465 * Rt + 17.343 \qquad \text{(Eq. 3)},$$

where *Lt* is the weight of the left otolith and *Rt* is the weight of the right one (N = 11, P < 0.001, R^2 = 0.99; Figure 15). The right otolith weights (N = 24) were then converted to the equivalent of the left using the model above. The combined otolith weights (*W*) were plotted against the observed and calculated fork lengths (*L*) (Figure 16).

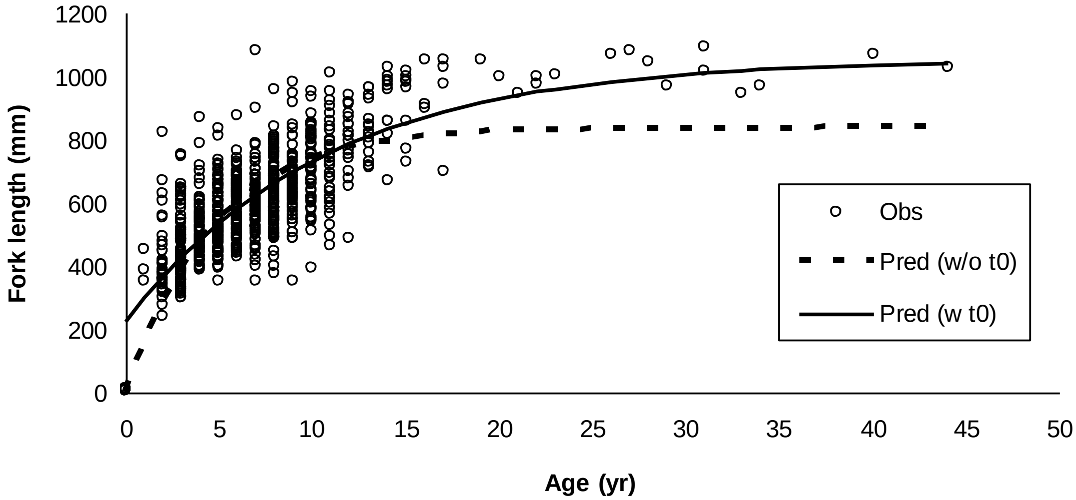


Figure 13. Von Bertalanffy growth curves fitted to the observed length at age data. Growth parameters are provided in Table 6, t_o = -0.25.

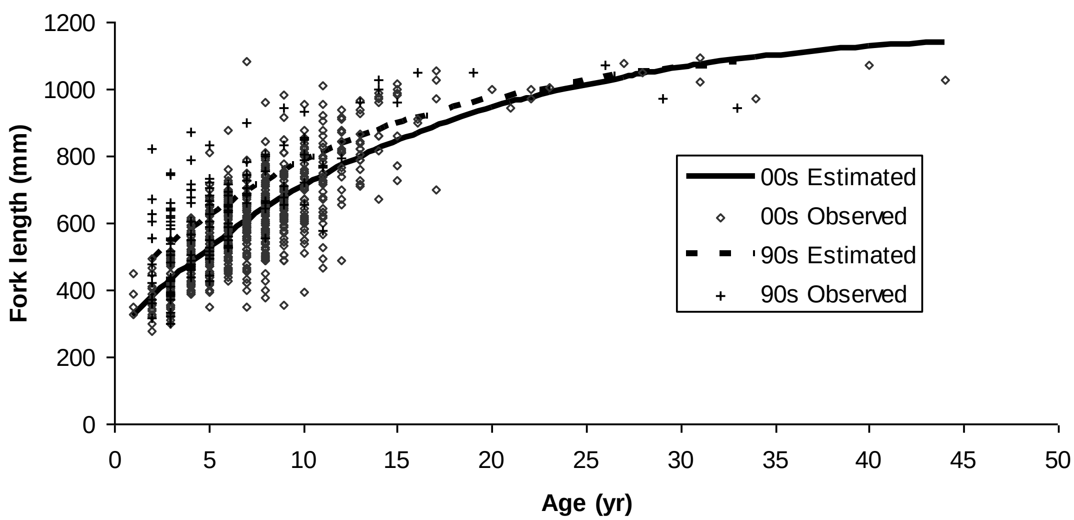


Figure 14 Bertalanffy growth curves fitted to the observed length at age data for the 1990s and 2000s.

Otolith weights, along with their corresponding ages are plotted in Figure 17. The otolith weights that did not correspond with the selected ageing precision criterion were not included in the analysis. The relationship between fish age and otolith weight was defined by the following linear model;

$$Age = 0.0172*W + 1.3377 \qquad \text{(Eq. 4)},$$

where *W* is otolith weight (N = 525, P < 0.001, R^2 = 0.73). It is evident from the model that otolith weight increases with age in snowy grouper. The model better represents younger fish (< 13 years). The weight of the otolith continued to increase with increasing age even in the older fish, but it does so at a slower rate.

Reproduction

The investigation of *E. nivetaus* reproduction was limited in this study by the small number of gonads collected during the study and the wide range of locations from which these fish were captured. From the total sample size of 92 gonads, 68 fish (73%) had otoliths that were assigned ages with agreement between readers of 0 – 1 years, the otoliths associated with 22 tissue samples (21%) were within an ageing precision of two – six years and one sample was assigned an age with an agreement of > six years difference. This portion of the study used an ageing precision of ≤ six years in order to incorporate the majority of the gonads collected and to provide an estimate of age at sexual transition.

Table 4. Growth parameter estimates of *E. niveatus*, using the von Bertalanffy growth model with estimated standard error and 95% confidence intervals. Standard errror for each parameter is in parentheses and the 95% CI are the lower and upper ranges, N is number, L_∞ is maximum length (mm), *k* is the growth coeffiecient, and t_0 is the theoretical age at which the fish would have zero length

		L_∞		K		t_0	
Period	**N**	**Mean Estimate (±) SE**	**95% CI**	**Estimate (±) SE**	**95% CI**	**Estimate (±) SE**	**95% CI**
1984-2004 no t_0	790	838 (14.5)	810 867	0.214 (0.009)	0.196 0.231		
1984-2004 $t_0 = 0$	790	1057 (39.0)	980 1134	0.094 (0.009)	0.077 0.111	-2.54 (0.334)	-3.19 -1.88
1984-2004 $t_0 = -0.25$	790	1057 (39.0)	980 1134	0.094 (0.009)	0.077 0.111	-2.54 (0.334)	-3.19 -1.88
1990-2004 $t_0 = -0.25$	770	1228 (71.9)	1086 1369	0.058 (0.008)	0.042 0.072	-5.23 (0.648)	-6.50 -3.96
1990s $t_0 = -0.25$	136	1147 (126)	897 1396	0.073 (0.023)	0.027 0.120	-5.60 (1.72)	-9.00 -2.20
2000-04 $t_0 = -0.25$	634	1194 (62.7)	1071 1317	0.065 (0.008)	0.050 0.081	-3.84 (0.528)	-4.88 -2.80

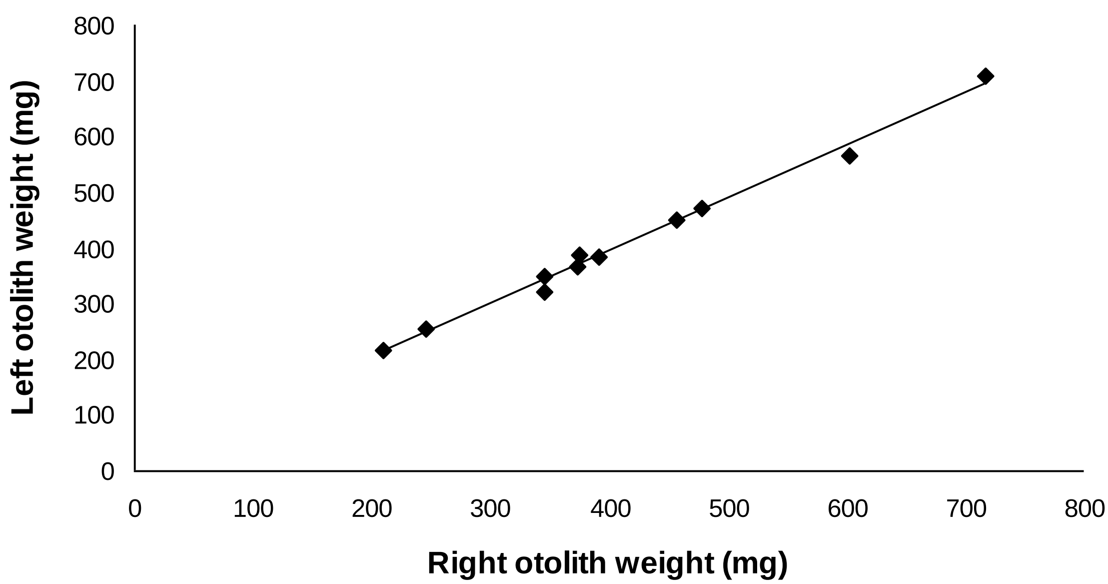


Figure 15. Relationship between the weight of right and left otoliths from *E. niveatus*. The following model: $L = 0.9465 * R + 17.343,$ where *L* is the left and *R* is the right (N = 11, P < 0.001, $R^2 = 0.99$).

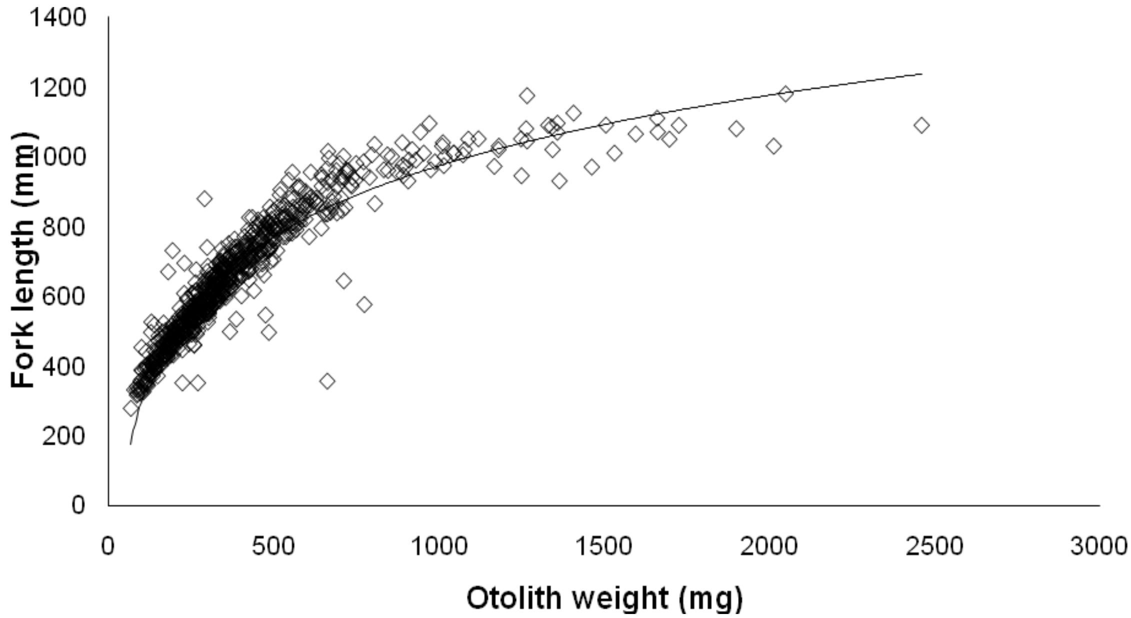


Figure 16. Logarithmic relationship between *E. niveatus* otolith weight and fork length. Otolith weight is *W* and fork length is *L*. The following model: *W = 291.9 * Ln(L) – 1042.4* (N = 741, R^2 = 0.90).

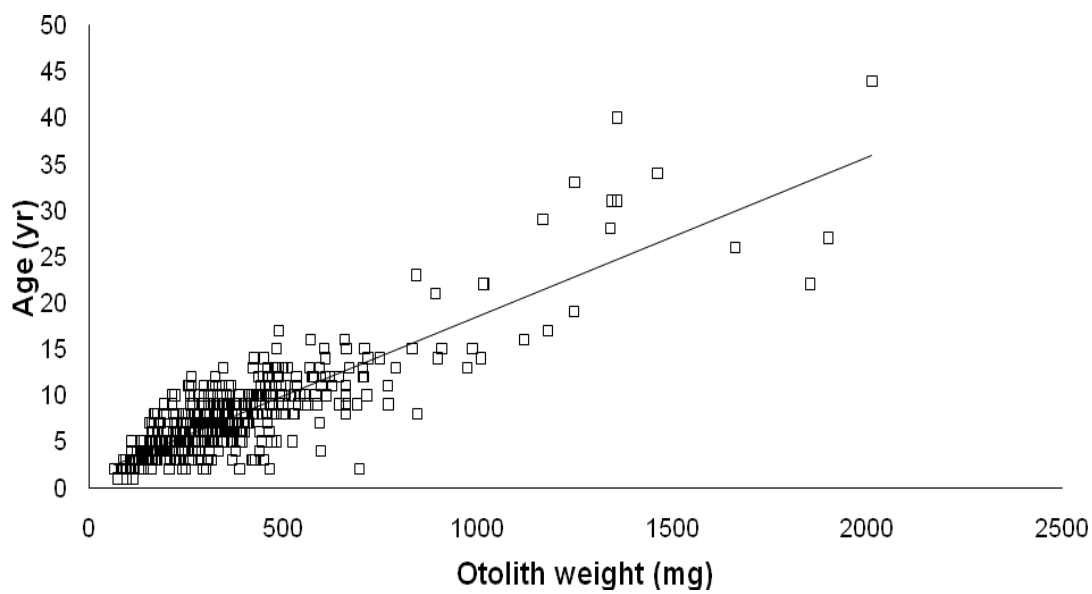


Figure 17. Linear relationship between *E. niveatus* otolith weight (*W*) (left and right combined) and age (*A*). The model that resulted was: *A = 0.0172*W + 1.3377* (*N* = 525, R^2 = 0.73).

The otoliths that had a reader disagreement of greater than two years were assigned ages by taking the average of the two ages and rounding down to the integer, as the primary reader tended to age higher.

Female snowy grouper made up the majority (91%, N = 82) of the samples used here, male fish represented a much smaller portion (3%, N = 3), and transitional fish accounted for 6% (N = 5). Two of the males and three of the transitional fish were included using the lower ageing precision. Females varied in age from three to 10 years, with fork lengths between 330 and 880 mm. The three males identified were 17, 20, and 27 years old, with fork lengths of 1,030, 955, and 1,080 mm, respectively. The five transitioning fish varied from 6 – 13 years with fork lengths from 474 to 930 mm.

The size, age, and month of occurrence of individuals in different reproductive states was variable (Table 5). Immature females comprised 7% (N = 6) of the total females collected. These young females were identified by short lamellae and the absence of complicated structure and musculature. They had only previtellogenic oocytes (primary growth), and were characterized by abundant cell nests along the periphery of the lamellae (Figures 18 and 19). Immature females were observed between three and five years of age, with fork lengths between 330 and 605 mm. Immature ovaries were collected during March, May, and July. Oocyte maturation was observed in 10% (N = 8) of the females (Figure 20). The fish were between 5 – 14 years old, with fork lengths between 551 and 822 mm. Mature oocytes were observed in fish collected in

April, May, July, September, and October. The majority of the gonads collected were from mature regressed females. Females with regressed ovaries comprised 56% (N = 48) of the all females and they were observed in all of the months sampled. These ovaries contained large lamellae, a structural component of the ovary, with a complicated, continuous structure (Figure 21). The ovaries contained many primary growth oocytes (prior to vitellogenesis or yolk formation), with one or more nucleoli (Figure 22). Some showed evidence of a previous spawn with atretic (degenerating) oocytes that were not released from the ovary. Five fish (6%), showed signs of previous spawning including postovulatory follicles (POF) (Figure 23), and atretic oocytes (Figure 24). Ovaries with evidence of spawning were identified in fish between five to nine years, with fork lengths between 497 and 755 mm. Post–spawn ovaries were observed during the months of March, May, September, and October. The remaining 17 (21%) female snowy grouper were categorized as ‘uncertain’ as the immature or regressed state of their ovaries could not be determined.

Snowy grouper, being protogynous hermaphrodites, were observed in varied states of transition. Three of the fish with transitional gonads were nearing the end of transition and their gonads were composed primarily of spermatozoa, while a few showed ongoing spermatogenesis. Two of the transitional fish identified had a portion of the gonad composed of ovary, while another section of the gonad was composed of testicular tissue (Figure 25). Transitional gonad tissue was observed in individuals collected during February, March, and June.

Transition from female to male begins in some snowy grouper between six and seven years of age and starting at fork lengths of approximately 475 mm, although much older fish were also found undergoing transition (12 and 13 years of age) at fork lengths greater than 900 mm.

Table 5. Number, size class (mm), and age (years) of fish in each reproductive state. N is number and FL is fork length (mm)

Reproductive State	N	smallest FL	largest FL	youngest	oldest	N with ageing difference	
						0 – 1 yr	<7 yrs
immature female	6	330	605	3	5	3	3
mature active female	8	551	822	5	14	6	2
post spawn female	5	497	755	5	9	4	1
mature regressed female	46	390	880	4	11	37	9
uncertain female	17	480	735	3	10	14	3
transitional	5	474	930	6	13	2	3
male	3	955	1080	17	25	1	2

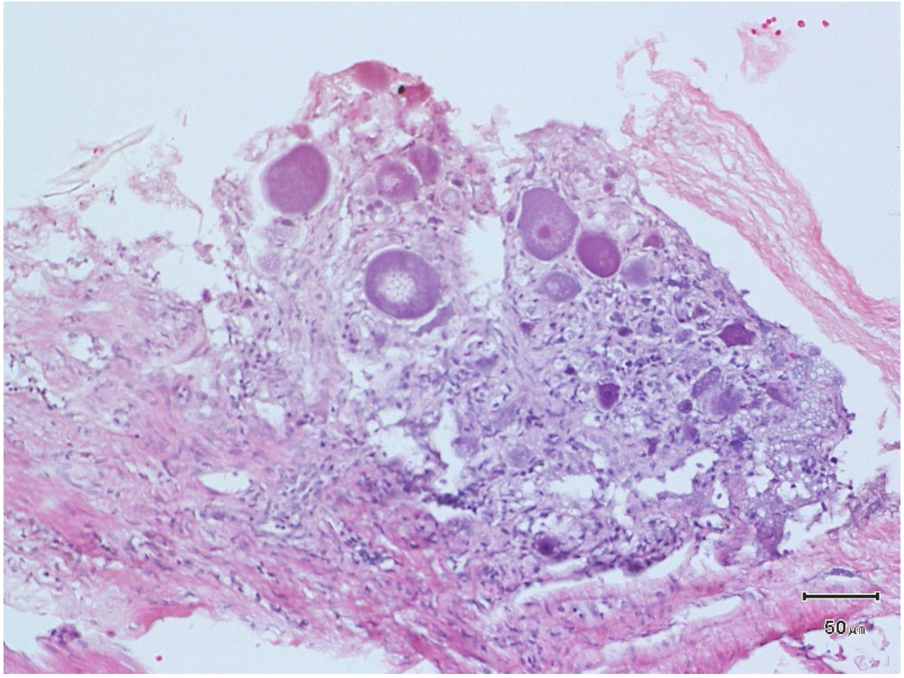


Figure 18. Section of ovary from an immature female *E. niveatus*. Only primary–growth oocytes and cell nests are shown; lamellae are structurally simple.

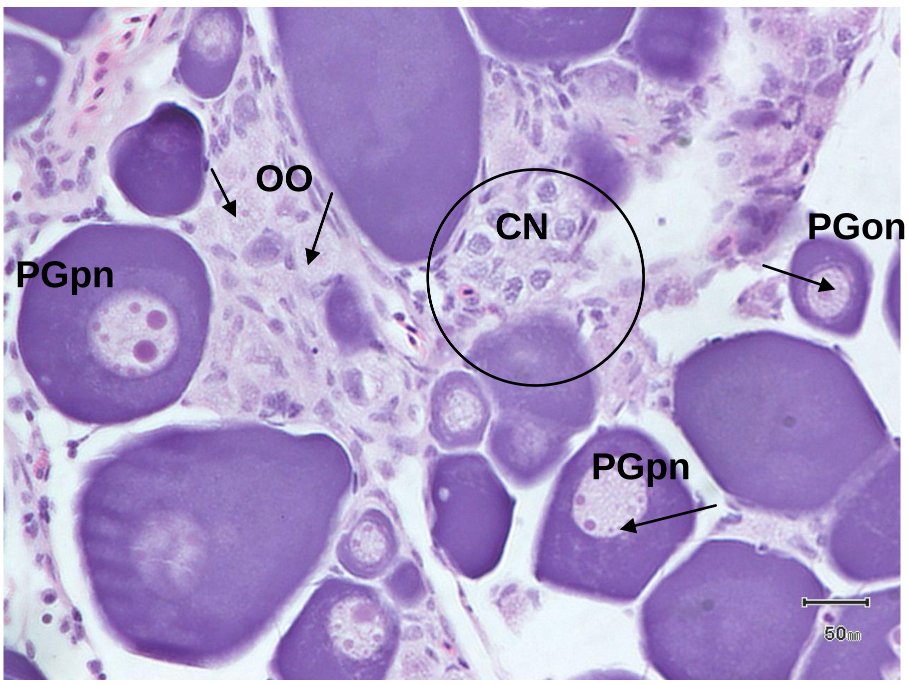


Figure 19. Immature female *E. niveatus* ovary with cells in various stages of chromatin nucleolus and primary–growth oocytes visible; oogonia (OO), primary–growth one nucleolus (PGon), primary–growth perinucleolar (PGpn), and cell nest (CN) labeled.

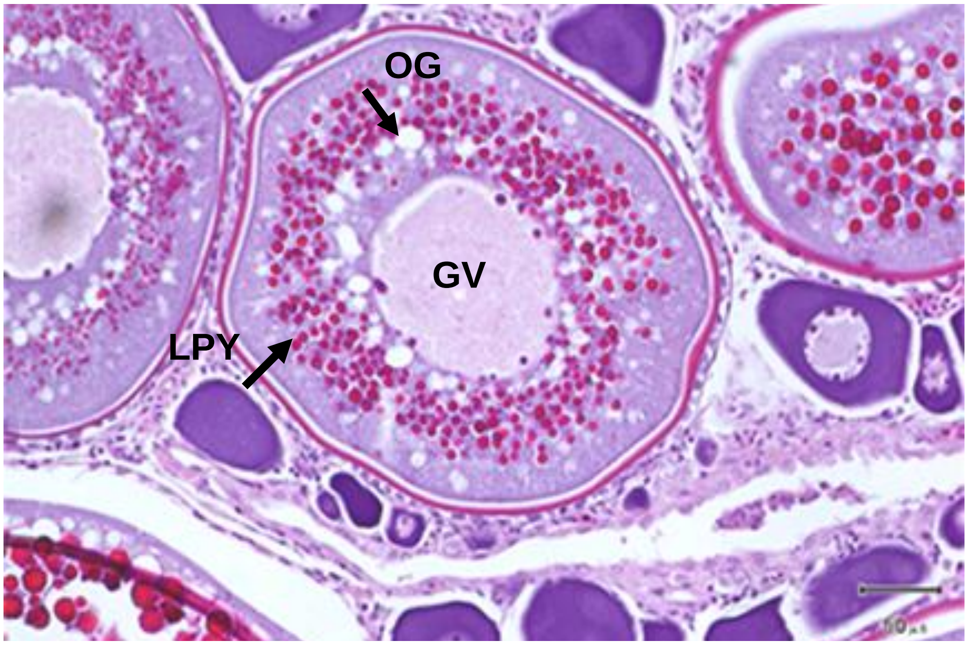


Figure 20. Maturing female *E. niveatus*. Early oocyte maturation with oil droplets forming globules (OG) around the germinal vesicle (GV). Lipoprotein yolk droplets (LPY) are also seen.

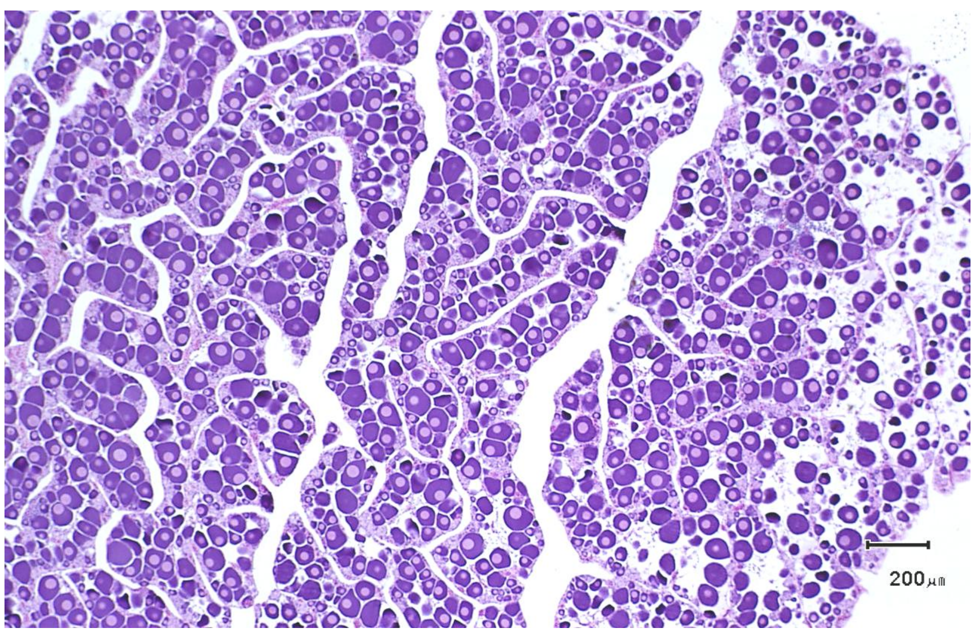


Figure 21. Example of extensive continuous structure of the lamellae found in mature regressed female *E. niveatus* ovaries.

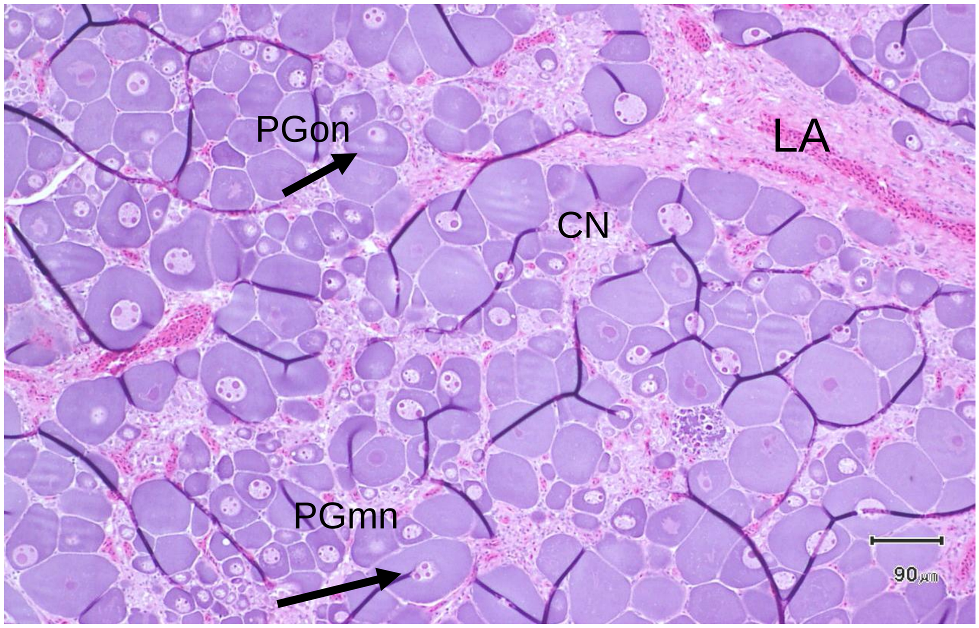


Figure 22. An example of different steps of primary–growth oocytes; including those with one nucleolus (PGon), multiple nucleoli (PGmn), and cell nests (primordial oogonia) (CN). The lamella are also labeled (LA).

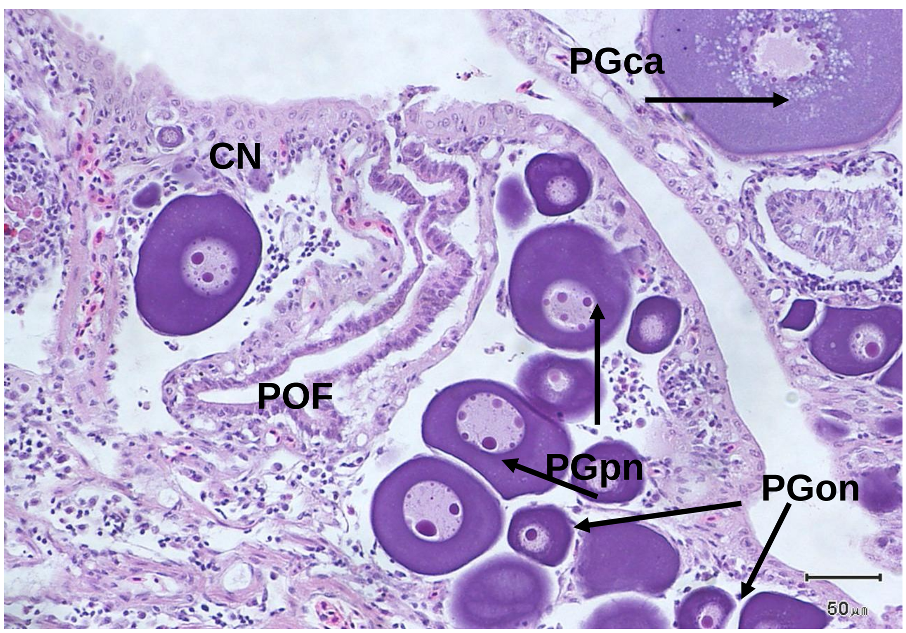


Figure 23. Post–ovulatory follicle (POF) showing the remains of the follicle cells after the oocyte has been released. A primary–growth oocyte in the cortical alveolar step (PGca) is seen on the upper right side. A cell nest (CN) and primary–growth oocytes in the one nucleolus and perinucleolar (PGpn) step are also seen here.

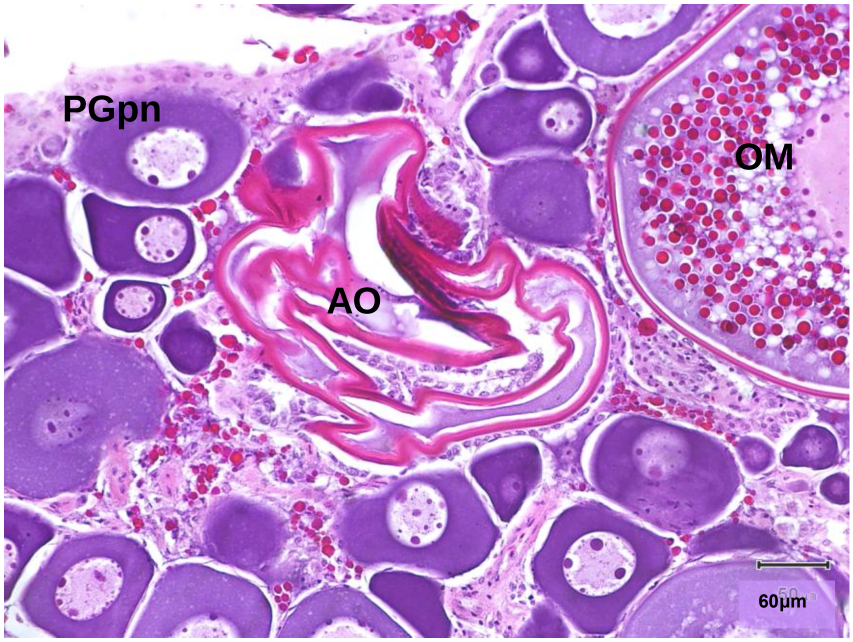
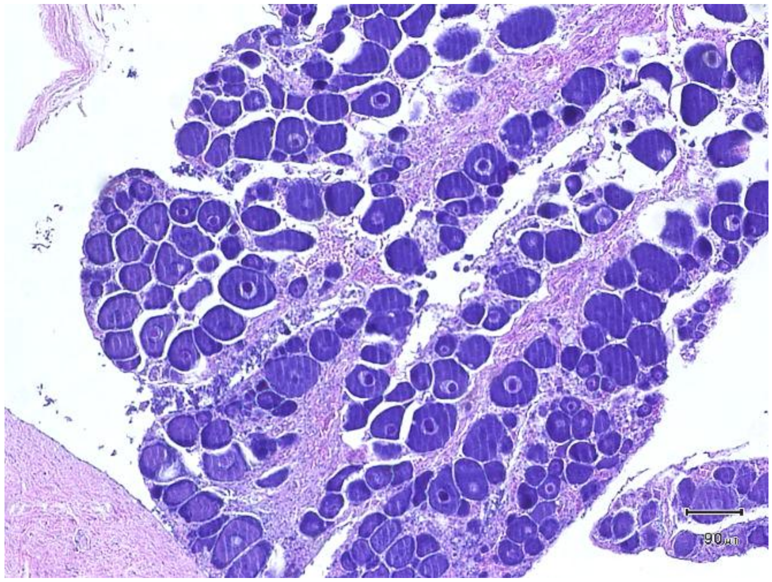


Figure 24. An atretic oocyte (degrading egg that won't be released, AO) surrounded by an oocyte in maturation (OM) on the right and primary growth perinucleolar oocytes (PGpn).

A



B

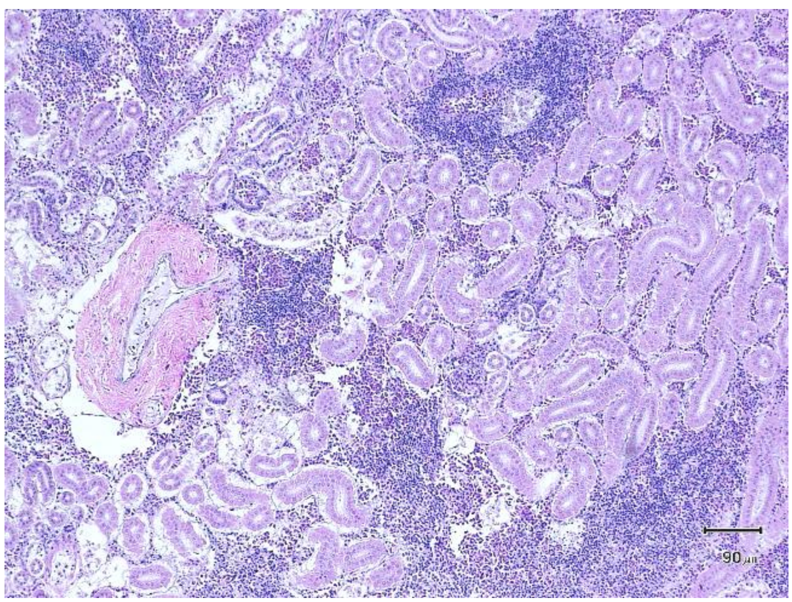


Figure 25. Transitional fish showing (A) ovarian tissue with evidence of primary growth and (B) immature testicular tissue. Both size bars represent 90 µm.

Discussion

Limitations of Fishery Sample Collection

The specimens from this study were acquired principally through commercial port sampling. Although the data provide an adequate representation of the fishery, they may not accurately represent the status of the snowy grouper within the Gulf of Mexico. Even though specimens were collected offshore of four different states, they could have come from anywhere along the continental shelf in the northern and eastern regions of the Gulf, where stocks likely share genetic material. Because the samples were commercially–derived, the ancillary information such as location, depth, water temperature, total weight, and sex were unavailable. A more consistent methodology of data collection including all of these variables would be advisable in the future. Finding methods to minimize variability would help reduce the need for measurement conversions and the associated error and provide larger sample sizes with multiple measurements to develop improved meristic relationships. In addition, collection of relevant environmental and location information would be desirable.

The snowy grouper from the Gulf of Mexico appear to enter the fishery at a young age, around two to three years of age, and are fully recruited by age five or six. The method of acquiring specimens from commercial and recreation vessels, as well as scientific sampling with longline gear, limits the samples to fish already being exploited by the fishery. Larval and young–of–the–year specimens were not collected, because of the modes fished and the gear used. Lack of data on larval size decreased the accuracy of the growth curves. To better manage the snowy grouper fishery, information is needed on the behavior and habitat preferences of this species, as well as determining the locations and dispersal patterns of recently hatched larvae, and the location of nursery grounds, which are most likely in shallower depths along the shelf (Moore and Labisky, 1984; Matheson and Huntsman, 1984).

The collected hard parts and tissue samples varied in quantity and quality from year to year. Many otoliths were broken, but still usable upon receipt, the result of either poor collection or handling in the small coin envelopes in which they were stored. The gonads, many of which were several years old, were fixed and stored principally in plastic bags, in which much of the formalin had leaked out over time. The larger gonad tissue samples showed evidence of poor fixation and preservation (often resembling fatty tissue). Unfortunately, the quantity of gonads available was low in comparison with the number of otoliths. Also, the lack of fully hydrated oocytes and a low number of males hindered a complete analysis of reproduction.

Age and Growth

Structures such as scales, fin rays, and otoliths have been used to determine the age of fish (Van Oosten, 1929; Pannella, 1971; Menon, 1959, cited in Jackson, 2007). The efficacy of otoliths for age determination is species dependent, although otoliths seem to be the form least susceptible to alterations in fish condition (Campana and Neilson, 1985). Previous studies of snowy grouper have used sectioned saggital otoliths for age assessment (Matheson and Huntsman, 1984; Moore and Labisky, 1984; Wyanski et al., 2000).

The use of otoliths, although far from optimal, is a suitable method for determining age in *E. niveatus*. The use of opaque zones to age *E. niveatus,* has been validated in previous studies (e.g., Matheson and Huntsman, 1984; Moore and Labisky, 1984; Wyanski et al., 2000). Otoliths from fish collected in the Gulf of Mexico can be aged at all sizes, although difficulties in interpretation exist throughout the entire age range. Ageing older fish tended to be more challenging and required considerably more time, but was not impossible. As noted, determination of annual increments was often impeded by various structures and formations, including excessive transparency, calcification, clouding, crowding, and changes of axis. The various problems with age determination resulted in a low initial agreement (41%). However, this low level compared well with the initial read percentage (24.2%) reported by Wyanski et al. (2000).

The age–length frequencies provided insights on the harvest of snowy grouper in the Gulf of Mexico. The collection of snowy grouper by commercial fishers remained low throughout the 1990s, and then between 2001 and 2004 the catch appeared increased. The increase in the catch of snowy grouper appears to be correlated with changes in the regulations of the shallow-water grouper complex. These regulatory changes may have lead to increased fishing pressure on the deep-water grouper species. Examination of year classes during the period of 2003 and 2004 reveal that year classes from 1997 and 1998 dominate the catch. The most recent *El Niño* occurred during the years of 1997 and 1998 and associations may have occurred between recruitment of snowy grouper and oceanographic cycles. Indirect relationships between oceanographic climate and recruitment of leopard grouper (*M. rosacea*) had been identified in the Gulf of California (Aburto-Oropez et al., 2007). Not only is it necessary to understand links between climate and fish abundance, but also the mechanisms by which climate influences biological changes within oceanographic ecosystems (Lehodey et al., 2006).

It should be noted that the appearance of strong year classes may be influenced by other unidentified variables, such as fishing method which may selectively favor age five and six snowy grouper. It has been reported that the snowy grouper harvested by the North Carolina and South Carolina longline fishery were principally (81%) aged one to six years, mostly female fish (Wyanski et al., 2000). We must first understand how the snowy grouper is influenced by the environment before we can fully understand how the impacts of humans on the species.

Seasonality of growth patterns was determined from margin codes (examining regions of slow growth) and these data validated that snowy grouper in the Gulf of Mexico deposit only one annulus per year. Also, this species deposited an opaque region on their otoliths once a year, peaking during spring and early summer, although opaque zone formation can begin as early as January. Thus, seasonal growth patterns of snowy grouper are similar within this geographic range. The results of this study agree with the results of previous studies of snowy grouper from offshore of the Carolinas and in the vicinity of the Florida Keys (Matheson and Huntsman, 1984, Moore and Labisky, 1984, Wyanski et al., 2000).

Growth parameters calculated for *E. niveatus* in this study are indicative of a relatively slow–growing, long–lived species, confirming previous findings (Matheson and Huntsman, 1984, Labisky and Moore, 1994, Wyanski et al., 2000). The von Bertalanffy growth model estimated a maximum length of 1,057 mm for the total aged sample of snowy grouper, with a maximum observed length of 1,096 mm. This estimate was reasonable based on the modeled observations; however, the largest fish that was unable to be aged was slightly larger at 1,190 mm, collected in October 2003. The L_∞ estimated from fish collected during the 2000s was 1,194 mm, much closer to the observed maximum length. Thus, the maximum size that a snowy grouper may obtain remains uncertain.

The Brody growth coefficient (k) shapes the growth curve as a measure of the rate at which the growth rate declines (Ricker, 1975). The higher the k value, the faster L_∞ is reached and the lower the value of L_∞ (Barry and Tegner, 1989). Estimated k values and the associated maximum lengths in this study were 0.094

and 1,057 mm for the pooled sample, 0.073 and 1,147 mm for the 1990s, and 0.065 and 1,194 mm for the 2000s. Snowy grouper was harvested at a greater frequency by the commercial fishing fleet, between 2000 and 2004, likely resulting from changes in federal regulation for shallow–water grouper fishing. These changes in the fishery may be reflected in the growth rates. The *k* value estimated for snowy grouper collected offshore of North Carolina and South Carolina during the late 1970s was 0.063 and the L_∞ was 1,255 mm total length (Matheson and Huntsman, 1984). Snowy grouper collected from the Florida Keys between 1978 and 1981, were estimated to have a *k* of 0.087 and the L_∞ was 1,320 mm (Labisky and Moore, 1984). The growth coefficients estimated for snowy grouper from the Gulf of Mexico are similar to those of other groupers from the Gulf of Mexico and the South Atlantic: red grouper, 0.11 and 1,026 mm (Lombardi-Carlson et al., 2002), speckled hind, 0.088 and 967 mm (Matheson and Huntman, 1984), and gag, 0.165 and 1,180 mm (Hood and Schlieder, 1992). The value derived from the growth coefficient from the pooled sample was a value greater for the two decades examined individually. The individual decades produced values nearer to those for the Carolinas and Florida Keys. Growth parameters defined in this study verify that snowy grouper growth in the Gulf of Mexico is similar to the growth of this species in other parts of the South Atlantic. Snowy grouper are slow–growing, reach maturity slowly (approximately 5 years old in this study), and tend to slow in growth considerably once they reach approximately 1,000 mm.

The relationship between otolith weight, age, and fork length were examined to determine whether otolith formation occurs in proportion to age and growth. Otolith weight was examined because otoliths easily break, making cross-section radius measurements difficult. In addition, snowy grouper are not easy to age and an alternative method to determine age would be useful in future work. Previous work by Matheson and Huntsman (1984) demonstrated a relationship between otolith radius, age, and body length in snowy grouper, suggesting this approach would be feasible.

Snowy grouper otoliths did accrete in proportion to their age and length. The relationship between otolith weight and fork length is best described as a semi-logarithmic relationship, which flattened out at fork lengths of approximately 1,000 mm and showed a significant amount of variation between 200 and 1,000 mm. The relationship between otolith weight and fish age was linear, indicating that otoliths continue to grow as the fish ages. The variation which occurred especially at the low range of ages (1 – 16 yrs), however, makes it difficult to use otolith weight to predict age. The low number of fish in the larger size and age classes, no doubt affects the model when dealing with fish over 16 years old.

The variation that exists between otolith weight, fish age, and length may result from environmental variability. Previous findings note that otoliths develop in isotopic equilibrium with the surrounding water (Devereux, 1967). Daily accretion in larval otoliths has been found to experience interruptions when fish are exposed to suboptimal conditions, which may include variations in light, temperature, and food availability (Jones, 1986). Pannella (1971) noted that deposition of daily rings may be associated with patterns of behavior, metabolism, and physiology. His findings also highlighted the fact that otoliths have an annual pattern of slow growth regions (annuli) composed of organic matter and fast growth regions (transparent) containing inorganic matter. Therefore, assuming that patterns of fast and slow growth can be influenced by conditions in which the fish reside during all periods of life it is reasonable older fish, with otolith changes in axis of deposition and crowding near the margin, had less accumulation during the fast–growth periods, more than likely due to the slowdown in overall growth.

Reproduction

Sex state was assigned to 90 specimens (8% of the specimens sampled), through histological analysis. Sex determination was limited because the majority of fish were sampled from the commercial fishery and the gonad had been removed when the fish were gutted. Samples consisted of 82 (91%) females, three (3.3%) males, and five (6%) transitional fish. Average female age and length was seven years and 609 mm. Mean age and length for males was 20.7 years and 1,022 mm. There were no males under 17 years or less than 955 mm fork length. The low number of gonad samples in relation to the number of otoliths used made it difficult to make any determinations regarding the significance of these findings. The aged sample contained fish both larger and older than those used for assigning gender.

Immature fish from the Gulf of Mexico were between three and five years old and ranged from 330 to 605 mm fork length. The youngest mature female in this study was five years old, with a fork length of 550 mm. Moore and Labisky (1984) found the youngest mature snowy grouper in the Florida Keys was three years old. Wyanski et al. (2000) identified the youngest mature female fish taken off the coasts of North and South Carolina as three year old. The current study did not have gonads from fish less than three years. Two three–year old fish were studied; one was classified as immature and the other was classified as either immature or regressed (im/rg). The fish classified as im/rg was collected in October and had a fork length of 508 mm, the immature three year old was collected in July and its length was 330 mm. Based on the size and date of capture the im/rg fish could have spawned in the months before capture. In North and South Carolina, immature fish were found up to nine years of age (Wyanski et al., 2000). The oldest immature fish found in the study from the Florida Keys was five years old (Moore and Labisky, 1984).

The transitioning specimens yielded two age groups (potentially the result of sampling methods): young transition (six and seven years old) and old transition (12 and 13 years). The fish that changed sex at a young age were smaller in size, between 474 and 645 mm. The fish that transitioned at older ages were larger; 915 and 930 mm. Most fish were in the later stages of transition, although one had male tissue on one part of the lobe and primary growth (one–nucleolus oocytes) on another portion. The transitioning fish were collected during February, March, and June. Moore and Labisky (1984) and Wyanski et. al. (2000) reported fewer fish at any stage of transition from the Florida Keys and the Carolinas. Gags have been found to transition within the first two or three months after spawning (McGovern et al., 1998); however, more data are needed to confirm this behavior for snowy grouper. The low number of transitional fish and males is similar to that seen in other exploited grouper populations such as gag and graysby (Huntsman and Schaff, 1994; Coleman et al., 1996; McGovern et al., 1998).

Snowy grouper in the Gulf of Mexico had females in spawning condition during April, May, July, September, and October. It is important to note that these females were collected from different areas of the Gulf. The fish found in spawning condition during April and May were taken from waters east of Louisiana and west of Madeira Beach, Florida. Specimens collected during late July, September, and late October were all from South Florida and the Florida Keys. Previous work indicated this species spawns between April and July in the Florida Keys (Moore and Labisky, 1984). Spawning fish were found April through July in the Carolinas, though the authors noted low sample sizes between October and March (Wyanski et al., 2000). Yellowedge grouper, *E. flavolimbatus*, were found in spawning condition between January and October, in the eastern Gulf of Mexico (Bullock et al., 1996). Timing of snowy grouper reproduction in the Gulf of Mexico is similar to that found in other regions of the south Atlantic, but more research is necessary to fully determine reproductive seasonality in the Gulf.

Size and structural complexity of ovarian lamellae appear to be an effective way to determine whether a fish has previously spawned. The mature-regressed ovaries possessed an intricate network of lamellae, with evidence of anastomosing (branching and coming back together). Regressed females sometimes had oocytes with coalescing oil droplets and the formation of cortical alveoli (vesicles), which surround the germinal vesicle prior to vitellogenesis (secondary growth) (Grier et al., 2009). In contrast, immature fish had short, small–branched lamellae, with many oogonia and cell nests.

A coordinated and inclusive analysis of snowy grouper reproduction is needed to be able to understand completely the biology of this species. Previous works provided information on the number and reproductive biology of females (Moore and Labisky, 1984, Wyanski et al., 2000), but little is known about the age, size, timing, and rate of transition in snowy grouper. Populations of snowy grouper appear to be composed largely of females (Moore and Labisky, 1984), having a broad range of sizes and ages. The reproductive season appears to be long, but comprehensive studies are lacking. In addition, the ratio of males to females has not been well documented in snowy groupers. Ghiselin (1969) presented early models which attempted explanations as to the reasoning for sexual transition; density dependent, size dependent, and in gene flow. Many attempts to model the biological, environmental, and physiological responses of sex change have followed (Armsworth, 2001; Muñoz and Warner, 2004; Davis and Berkson, 2006). The ability of the fishery to overcome exploitation may be dependent upon the ability of the fish to compensate for loss of males and a limitation of sperm; which means a lot of information is still needed to adequately manage protogynous fish species (Huntsman and Schaff, 1994). Size selective fisheries may have unseen consequences effecting the sex ratio, spawning behavior, and fecundity (Alonzo and Mangel, 2003). Increased fishing pressure may be associated with the low number of transitional and male fish or it may be the result of behavior or habitat preference. This study highlights the need for further work on all aspects of the life history of snowy grouper.

Conclusion

While *Epinephelus niveatus* is a valuable commercial fishery throughout the Gulf of Mexico, the status and condition of the fished stocks has yet to be determined. Most studies of the life histories of other groupers tend to be limited to shallow–water species. As the Gulf of Mexico grouper fishery becomes more exploited over time, the likelihood that deep–water species will experience greater fishing pressure is high. Maintaining healthy grouper fisheries is essential and may require changes in current management paradigms, especially to accommodate species such as the snowy grouper, which is unlikely to be successfully managed using current approaches such as size and bag limits. The present research provided valuable life history information on the age and growth, reproduction, and sex change that can be used to help manage fisheries and aid in future stock assessment models.

Information regarding the life histories of hermaphroditic species is essential because overfishing impacts them differently than species that do not experience a change in sex (gonocharistic) (Moore and Labisky, 1984; Huntsman and Schaaf 1994; Wyanski et al., 2000; Brooks et al. 2008). Combined with the nature of slow growth rate, late maturity, and little known behavior patterns, the fact that snowy grouper change sex complicates the management picture and makes the threat of overexploitation even greater. Many stock assessment models require a multitude of life history parameters to be successful and those with limited information should be used with caution. Although it is difficult to predict the life history patterns of fish that are challenging to study, it emphasizes the need for more complete data on the age and growth, reproduction, and spawning behavior of such species. The recent oil spill in the Gulf of Mexico has also helped the scientific community to focus on the need for more robust and complete life history information on commercially and recreationally valuable fish species, to understand better the impacts of both natural and human–induced events

The present research is the first to examine aspects of the life history of the snowy grouper in the Gulf of Mexico and highlights the need for more complete studies on not only this species, but also on many other valuable deep–water fish species. Ecosystem–based studies, using targeted sampling and collection of important data, such as depth, bottom type, temperature, sexual condition, and diet, are essential to improve understanding of the developmental and behavioral patterns of many fish species in the Gulf of Mexico.

Works Cited

Aburto-Oropeza, O., E. Sala, G. Paredes, A. Mendoza, and E. Ballesteros. 2007. Predictability of reef fish recruitment in a highly variable nursery habitat. Ecology. 88: 2220 – 2228.

Alonzo, S. H. and M. Mangel. 2004. The effects of size-selective fisheries on the stock dynamics of and sperm limitation in sex-changing fish. Fish. Bull. 102: 1 - 13.

Armsworth, P. R. 2001. Effects of fishing on a protogynous hermaphrodite. Can. J. Fish. Aquat. Sci. 58: 568 – 578.

Barry, J. P. and M. J. Tegner. 1989. Inferring demographic processes from size-frequency distributions: simple models indicate specific patterns of growth and mortality. Fish. Bull. 88: 13 - 19.

Bartoo, N. W. and K. R. Parker. 1983. Stochastic age-frequency estimation using the von Bertalanffy growth equation. Fish. Bull. 81: 91 – 96.

Bohlke, J. E. and C. G. Chaplin. 1968. Fishes of the Bahamas and adjacent tropical waters. Livingston Press. 771 p.

Bielsa, L.M. and R.F. Labisky. 1987. Food habits of blueline tilefish, *Caulolatilus microps*, and snowy grouper, *Epinephelus niveatus*, from the lower Florida Keys. Northeast Gulf Sci. 9: 77 – 87.

Brooks, E. N., K. W. Shertzer, T. Gedamke, and D. S. Vaughan. 2008. Stock assessment of protogynous fish: evaluating measures of spawning biomass used to estimate biological reference points. Fish. Bull. 106: 12 – 23.

Bullock, L. H., M. F. Godcharles, and R. E. Crabtree. 1996. Reproduction of yellowegde grouper, *Epinphelus flavolimatus*, from the eastern Gulf of Mexico. Bull. Mar. Sci. 59: 216 – 224.

Campana, S. E. and J. D. Neilson. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences. 42: 1014 – 1032.

Campana, S. E. and C. M. Jones.1992. Analysis of otolith microstructure data. Pages 73–100 in Stephens, D. K. and S. E. Campana, eds. *Otolith microstructure examination and analysis*. Can. Spec. Publ. Fish. Aquat. Sci. 117.

Carpenter, K. E. (ed.). 2002. *The Living Marine Resources of the Western Atlantic. Volume 2: Bony fishes part 1 (Acipenseridae to Grammatidea)*. Food and Agriculture Org. of the United Nations. Rome, Italy. 1373 p.

Craig, M. T. and P. A, Hastings. 2007. A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with revised classification of the epinephelini. Ichthyol. Res. 54: 1 – 17.

Coleman, F. C., C. C. Koenig, and L. A. Collins. 1996. Reproductive styles of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environ. Biol. Fish. 47: 129 – 141.

Davis, M. L. and J. Berkson. 2006. Effects of a simulated fishing moratorium on the stock assessment of red porgy (*Pagrus pagrus*). Fish. Bull. 104: 585 – 592.

Devereux, I. 1967. Temperature measurements from oxygen isotope ratios of fish otoltihs. Science. 155: 1684 – 1685.

Fitch, J. E. and S. A. Schultz.1978. Some rare and unusual occurences of fishes off California and Baja California. California Fish and Game. 64: 74 - 92.

Ghiselin, M. T. 1969. The evolution of hermaphroditism among animals. Q. Rev. Biol. 44: 189 – 208.

Grier, H. J., M. C. Uribe Aranzábal, and R. Patiño. 2009. The ovary, folliculogenesis, and oogenesis in teleosts. *In: Reproductive Biology and Phylogeny of Fishes*, Chapter 2 (Jamieson, B. G. M., ed.), Volume 8A. Science Publishers, Enfield, New Hampshire, USA.

Hood, P. B. and R. A. Schlieder. 1992. Age, growth, and reproduction of gag, *Mycteroperca microlepis*, (Pisces: Serranidae), in the eastern Gulf of Mexico. Bull. Mar. Sci. 51: 337 – 352.

Hunter, J. R. and S. R. Goldberg. 1980. Spawning incidence and batch fecundity in northern anchovy, *Engraulis mordax*. Fish. Bull. 77: 641 – 652.

Hunter, J. R. and B. J. Macewicz. 1985. Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax*. Fish. Bull. 83: 119 – 16.

Huntsman, G.R. and R.L. Dixon. 1976. Recreational catches of four species of groupers in the Carolina headboat fishery. Proceedings of the Annual Conference Southeastern Association of Game and Fish Commissioners. 29: 185 – 194.

Huntsman, G. R. and W. E. Schaaf. 1994. Simulation of the impact of fishing on reproduction of a protogynous grouper, the graysby. N. Am. J. Fish. Manag. 14: 41 – 52.

Jackson, J. R. 2007. Earliest references to age determination of fisheries and their early application to the study of fisheries. Fisheries. 32: 321 – 328.

Jagadis, I., B. Ignatius, D. Kandasami, M. A. Khan. 2006. Embryonic and larval development of honeycomb grouper *Epinephelus merra* Bloch. Aquaculture Res. 37: 1140 – 1145.

Jerald, A., Jr. 1983. Age determination. Pp 301 - 324 *In*: Nielsen, L.A. and D.L. Johnson (eds.), *Fisheries Techniques*. Am. Fish. Soc. Bethesda, Maryland. USA.

Jones, C. 1986. Determining age of larval fish with the otolith increment technique. Fish. Bull. 84: 91 – 103.

Knaggs, E. H., J. S. Sunada, and R. N. Lea. 1975. Notes on some fishes collected off the outer coast of Baja California. California Fish and Game 61: 56 – 59.

Lehodey, P., J. Alheit, M. Barange, T. Baumgartner, G. Beaugrand, K. Drinkwater, J.-M. Fromentin, S. R. Hare, G. Ottersen, R. I. Perry, C. Roy, C. D. van der Lingen, and F. Werner. 2006. Climate variability, fish, and fisheries. J. of Climate (Sp. sec.). 19: 5009 – 5030.

Lombardi-Carlson, L. A., G. R. Fitzhugh, and J. J. Mikulas. 2002. *Red grouper (Epinephelus morio) Age–Length structure and Description of Growth from the Western Gulf of Mexico: 1992-2001*. National Marine Fisheries Service, Southeast Fisheries Science Center. Panama City Laboratory Contribution Series 2002 – 2006.

Low, R. A., and G. F. Ulrich. 1983. *Deep-water Demersal Finfish Resources and Fisheries off South Carolina*. So.Car. Mar. Resour. Center Tech. Rep. 57, 24 p.

Manly, B. F. J. 2007. *Randomization, Bootstrap and Monte Carlo Nethods in Biology*, (3rd ed.) Chapman & Hall/CRC, Boca Raton, FL, 455 p.

Matheson, R.H., III, and G.R. Huntsman. 1984. Growth, mortality, and yield-per-recruit models for speckled hind and snowy grouper from the United States South Atlantic Bight. Trans. Am. Fish. Soc. 113: 607 – 616.

McGovern, J. C., D. M. Wyanski, O. Pashuk, C. S. Manooch, III, and G. R. Sedberry. 1998. Changes in the sex ratio and size at maturity of gag, *Mycteroperca microlepis*, from the Atlantic coast of the southeastern United States during 1976–1995. Fish. Bull. 96: 797 – 807.

Moe, M. A., Jr. 1969. Biology of the red grouper, *Epinephelus morio* (Valenciennes, 1828), from the Florida straits. Florida Dep. Nat. Resour. Marine Research Laboratory Professional Paper Series 10, 95 p.

Molina, S. 1999. *User's Guide for the TIP Trip Interview Program*. National Marine Fisheries Service, Fisheries Science Center. Miami, FL.

Moore, C. M. and R. F. Labisky. 1984. Population of a relatively unexploited stock of snowy grouper in the lower Florida Keys. Trans. Am. Fish. Soc. 113: 322 – 329.

Miller, D. J. and R. N. Lea. 1976. *Guide to the Coastal Marine Fishes of California*. California Dept. Fish and Game Fish. Bull. 157.

Muñoz, R. C. and R. R. Warner. 2004. Testing a new version of the size-advantage hypothesis for sex change: sperm competition and size skew effects in the bucktooth parrotfish, *Sparisoma radians*. Behavioral Eco. 15: 129 – 136.

Nickum, J. G., H. L. Bart Jr., P. R. Bowser, I. E. Greer, C. Hubbs, J. A. Jenkins, J. R. MacMillan, F. W. Rachlin, R. D. Rose, P. W. Sorensen, and J. R. Tomasso. 2004. Guidelines for the use of fishes in research. American Fisheries Society, American Society of Ichthyologists and Herpetologists, and the American Institute of Fishery Research Biologists. Available online at www.fisheries.org/html/Public_Affairs/Sound_Science/Guidelines2004.shtml.

Pannella, G. 1971. Fish otoliths: daily growth layers and periodical patterns. *Science* 173: 1124 – 1127.

Parker, R. O., Jr., and R.W. Mays. 1998. *Southeastern United States Deepwater Reef Fish Assemblages, Habitat Characteristics, Catches, and Life History Summaries*. U.S. Dept. Commer., NOAA Tech. Rep. NMFS 138, 41 p.

Presley, R. F. 1970. *Larval Snowy Grouper, Epinephelus niveatus, (Valenciennes, 1828), From the Florida Straits*. FL. DNR Leaflet Series IV: 6 p.

Ricker, W. E. 1975. *Computation and Interpretation of Biological Statistics of Fish Populations*. Fisheries Research Board of Canada, Bulletin 191, 382 p.

Roe. R. B. 1976. Distributions of snappers and groupers in the Gulf of Mexico and Caribbean Sea as determined from exploratory fishing data. p 129 -164 *in* Bullis, H. R., Jr., and A. C. Jones, eds., *Proceedings: colloquium on snapper-grouper fishery resources of the western central Atlantic Ocean*. FL Sea Grant Prog., Report 17, Gainesville, FL.

SAS Institute Inc. 2003. SAS/STAT® 9.1 User's Guide. Cary, NC: SAS Institute Inc.

Smith, C. L. 1971. A revision of the American groupers: *Epinephelus* and allied genera. Bull. Am. Mus. Nat. Hist. 146: 228 – 241.

Tucker, J. W. Jr. 1999. *Species Profile Grouper Aquaculture*. Southern Regional Aquaculture Publication No. 721.

Wenner, C. A., W. A. Roumillat, and C. W. Waltz. 1986. Contributions to the life history of black sea bass, *Centropristis striata*, off the southeastern United States. Fish. Bull. 84: 723 – 741.

Wyanski, D. M., D. B. White, and C. A. Barans. 2000. Growth, population age structure, and aspects of the reproductive biology of snowy grouper, *Epinephelus niveatus*, off North Carolina and South Carolina. Fish. Bull. 98: 199 – 218.

Van Oosten, J. 1929. Life history of the lake herring of Lake Huron as revealed by its scales, with a critique of the scale method. Bulletin of the Bureau of Fisheries XLIV: 267 – 417.

VanderKooy, S. (ed.) 2009. *A Practical Handbook for Determining the Ages of Gulf of Mexico Fishes*. (2nd ed.) Gulf States Marine Fisheries Commission. Publication Number 111. Ocean Springs, MS.

Websites Cited

FWRI web (Florida Fish and Wildlife Research Institute commercial landings page) visited 10/05/2010 = http://research.myfwc.com/features/view_article.asp?id=19224

RIGZONE 2010 (oil industry online newsletter) visited 10/05/2010 = http://www.rigzone.com/news/article.asp?a_id=79913

Appendix A

Supplementary Tables

Table A1 Age and length-frequency of *E. niveatus* in the Gulf of Mexico. Frequencies represented in number of fish per age group and 50 mm fork length (FL) intervals (1984 – 2004), N is number

Age (yr)		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	19	20	21	22	23	26	27	28	29	31	33	34	40	44
FL (mm)	N																															
200	1		1																													
250	2		1	1																												
300	16	1	6	9																												
350	40	2	11	15	5	2		1	2	1	1																					
400	52		5	12	16	12	2	4	1																							
450	87	1	4	12	26	17	11	5	5	3		2	1																			
500	93			8	11	24	18	13	10	6	2	1																				
550	97		2	5	10	14	19	21	14	5	5	2																				
600	97		2	9	5	11	16	17	10	14	7	6																				
650	85		1	1	2	9	20	14	14	12	6	3	2		1																	
700	61			1	3	5	6	6	9	10	9	5	2	3		1		1														
750	39			1	1		1	3	9	3	8	5	3	4		1																
800	36		1			2			4	5	8	4	6	5	1																	
850	15				1		1				5	2	2	1	2	1																
900	15							1		2	1	2	4	2			2												1			
950	18								1	1	1	1		2	4	3		1			1	1					1			1		
1000	11											1			2	2		1		1		1	1					1				1
1050	9							1									1	1	1					1	1	1		1			1	
Totals	774	4	34	74	80	96	94	86	79	62	53	34	20	17	10	8	3	4	1	1	1	2	1	1	1	1	1	2	1	1	1	1

Table A2. Age-length key for *E. niveatus* collected during 1991. Frequencies in proportions; abbreviations as in Table A1

Age (yrs)		2	3	4	5	6	7	9	10	13	16	26	29
FL (mm)	N												
300	1		1.00										
350	2	0.50	0.50										
400	3	0.33	0.67										
450	2	0.50		0.50									
500	7		0.43	0.29		0.29							
550	7	0.29	0.57	0.14									
600	17	0.12	0.53	0.18	0.12	0.06							
650	10	0.10	0.10	0.20	0.30	0.10	0.10		0.10				
700	10		0.10	0.30	0.20	0.10	0.20	0.10					
750	2		0.50	0.50									
800	3	0.33			0.33			0.33					
850	2			0.50					0.50				
900	2							0.50	0.50				
950	2									0.50			0.50
1050	2										0.50	0.50	
Total	72	9	23	14	8	5	3	3	3	1	1	1	1

Table A3. Age-length key for *E. niveatus* collected during 1992. Frequencies in proportions

Age (yrs)		2	3	4	5	6	7	8	9	10	11	12	15	19
FL (mm)	N													
300	1		1.0											
350	4	0.25	0.75											
400	1			1.0										
450	2			1.0										
500	4				0.25	0.75								
550	0													
600	1							1.0						
650	4					0.25		0.25	0.5					
700	2						0.5			0.5				
750	4							0.25			0.5	0.25		
800	2							0.5		0.5				
950	1												1.0	
1000	0													
1050	1													1.0
Total	27	1	4	3	1	4	1	4	2	2	2	1	1	1

Table A4. Age-length key for *E. niveatus* collected during 1999. Frequencies in proportions

Age (yrs)		4	5	6	7	8	9	10	11
FL (mm)	N								
400	2		2.00						
450	1		1.00						
500	2		1.00						
550	5	0.20	0.40	0.20					0.20
600	0								
650	2			0.50	0.50				
700	0								
750	1				1.00				
800	1					1.00			
850	0								
900	1				1.00				
Total	15	1	7	2	3	1	0	0	1

Table A5. Age-length key for *E. niveatus* collected during 2000. Frequencies in proportions

Age (yrs)		5	6	7	8	9	10	11	12	13	14	15
FL (mm)	n											
500	2	0.50	0.50									
550	0											
600	1		1.0									
650	0											
700	2						0.50			0.50		
750	0											
800	1								1.0			
850	0											
900	0											
950	2				0.50	0.50						
1000	2							0.50				0.50
Total	10	1	2	0	1	1	1	1	1	1	0	1

Table A6. Age and length key for *E. niveatus* collected during 2001. Frequencies in proportions

Age (yrs)		1	2	3	4	5	6	7	8	9	10	12	14	15	31	34
FL (mm)	N															
300	2	0.5		0.5												
350	4	0.5		0.25	0.25											
400	4		0.25	0.25	0.5											
450	8		0.13	0.25	0.25	0.25	0.13									
500	11				0.09	0.36	0.45			0.09						
550	6			0.17	0.17	0.17		0.33	0.17							
600	6				0.17	0.17	0.17	0.17		0.17	0.17					
650	8					0.25	0.13	0.13	0.13	0.13	0.25					
700	3								0.67	0.33						
750	1								1.0							
800	2					0.5						0.5				
850	0															
900	0															
950	3												0.33	0.33		0.33
1000	1														1	
Total	59	3	2	6	8	11	8	4	5	4	3	1	1	1	1	1

Table A7. Age-length key for *E. niveatus* collected during 2002. Frequencies in proportions

Age (yrs)		2	3	4	5	6	7	8	9	10	12	14	15	40
FL (mm)	N													
250	1	1												
300	4	0.5	0.5											
350	5	0.2	0.6	0.2										
400	5	0.2	0.4	0.4										
450	8	0.1	0.3	0.4	0.1	0.1								
500	8			0.4	0.3	0.1	0.1			0.1				
550	8			0.1	0.1	0.3	0.1	0.4						
600	2				0.5		0.5							
650	0													
700	2								0.5				0.5	
750	3					0.3				0.3	0.3			
800	2									1				
850	2									0.5		0.5		
900	1										1			
950	1											1		
1000	2												0.5	0.5
Total	54	6	9	10	5	5	3	3	1	5	2	2	2	1

Table A8. Age-length key for *E. niveatus* collected during 2003. Frequencies in proportions

Age (yrs)		1	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	20	22	23	27	28
FL (mm)	N																					
350	8		0.5	0.13	0.25					0.13												
400	21		0.05	0.38	0.43	0.05	0.1															
450	32	0.03	0.09	0.28	0.19	0.19	0.09	0.03	0.03		0.03	0.03										
500	23		0.04		0.13	0.09	0.22	0.3	0.17		0.04											
550	28			0.04	0.14	0.21	0.25	0.21	0.11	0.04												
600	30			0.03	0.03	0.13	0.17	0.17	0.23	0.1	0.1											
650	16					0.25	0.19	0.13	0.19	0.13	0.06	0.06										
700	11					0.18		0.18	0.18	0.27	0.09		0.09									
750	6						0.17	0.33	0.17	0.17					0.17							
800	6							0.17		0.17		0.17	0.33	0.17								
850	5									0.2	0.2	0.4		0.2								
900	8								0.13		0.25	0.25	0.13			0.25						
950	5										0.2			0.4	0.2		0.2					
1000	3																	0.33	0.33	0.33		
1050	4						0.25										0.25				0.25	0.25
Total	206	1	9	20	25	25	27	27	22	13	11	7	4	4	2	2	2	1	1	1	1	1

Table A9. Age-length frequency for *E. niveatus* collected during 2004. Frequencies in proportions

Age (yr)		2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	21	22	31	44
FL (mm)	N																			
300	6	0.5	0.5																	
350	14	0.36	0.21	0.14			0.07	0.14	0.07											
400	13	0.08	0.04	0.23	0.08	0.08	0.15	0.08												
450	31	0.03	0.13	0.23	0.23	0.1	0.06	0.13	0.06		0.03									
500	33		0.09	0.15	0.3	0.12	0.21	0.06	0.03	0.03										
550	41			0.12	0.15	0.2	0.27	0.1	0.05	0.1	0.02									
600	39				0.15	0.21	0.26	0.08	0.15	0.08	0.08									
650	44				0.09	0.25	0.16	0.2	0.14	0.07	0.05	0.02		0.02						
700	29				0.07	0.1	0.1	0.17	0.17	0.1	4	0.07	0.03			0.03				
750	20						0.05	0.25	0.1	0.2	0.15	0.05	0.2							
800	19							0.05	0.21	0.21	0.21	0.16	0.16							
850	5					0.2				0.2	0.2		0.2		0.2					
900	3											0.33	0.33				0.33			
950	3									0.33			0.33					0.33		
1000	2															0.5				0.5
1050	1																		1	
Total	303	10	17	22	36	39	44	36	29	24	19	8	11	1	1	2	1	1	1	1