Abstract

ADAMSKI, KYLE MICHAEL. Developing an Index of Abundance for Gag Grouper (*Mycteroperca microlepis*) in North Carolina. (Under the direction of Dr. Jeffrey A. Buckel.)

Gag grouper (*Mycteroperca microlepis*) are an economically important fish in the U.S. south Atlantic. The most recent stock assessment for gag grouper indicated the need for a fishery-independent index of abundance. The goal of this study was to assess the potential for a post-larval or juvenile abundance index in North Carolina. Data on post-larval gag collected during the NOAA Beaufort Inlet Bridgenet Program (weekly samples from November – May of 1986 to 2008) were examined; additionally, ichthyoplankton were sampled nightly in spring of 2007 and 2008. Juvenile gag grouper utilize seagrass habitat soon after larval ingress into estuarine environments. A 5-m otter trawl was used to sample juvenile gag in seagrass beds at 15 to 20 randomly selected stations every two weeks from June through September in 2007 and 2008; data on seagrass species and blade densities were determined before each trawl. Age at capture, pelagic larval duration (PLD, determined from transition mark), and fertilization dates were estimated from post-larval and juvenile otolith microstructure. A single cohort was produced each year; estimated fertilization dates ranged from February through April around full and new moons supporting planned January through April fishing closures for adult gag grouper. From 1986 to 2008, weekly concentrations of post-larval gag grouper were highest from late April to mid-May with peak ingress around new moons. Juvenile gag were caught from June through September with highest catch-per-unit-effort (CPUE) in July and August. Time of year, percent seagrass coverage, seagrass species, and sound influenced CPUE of juvenile gag grouper. Growth rates of juveniles were rapid (~1.6 mm/d) during summer months and did not differ between years. The mean PLD

was ~ 43 d and did not differ among collection months suggesting no effect of PLD on survival. An annual index of post-larval abundance (adjusted for lunar effects) was developed. The spawning stock biomass (SSB) from the most recent gag grouper assessment was positively correlated with this index; thus, the post-larval index could be used as a fishery-independent index of SSB.

Developing an Index of Abundance for Gag Grouper (*Mycteroperca microlepis*) in North Carolina

by

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Biography

Kyle Adamski was born in Greenfield, MA on January 16, 1982. Growing up in the Connecticut River basin he spent his time fishing mostly in freshwater and playing sports. Entering college as a sport management major and playing on the baseball team, injuries soon forced him to focus more on academics and less on a future in baseball. The summer of his freshman year he became a PADI certified Advanced Open Water scuba diver. Seeing the underwater environment more intimately than with just a hook and line, he developed a passion for understanding and preserving America's "Cast" Time. The following summer he took a trip to Beaufort, NC to dive on the "Graveyard of the Atlantic". As an undergraduate at UMASS – Amherst, he spent a semester abroad in Australia gaining a more global perspective on fisheries. After graduating with a B.S. in Biology, he worked in a variety of temporary jobs as a fisheries technician and in the aquaculture of tilapia and barramundi. He was fortunate enough to get into graduate school at NCSU and conduct his research back on the Crystal Coast.

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DEVELOPING AN INDEX OF ABUNDANCE FOR GAG GROUPER IN NORTH CAROLINA

1.1 Introduction

Recruitment of juveniles to the adult population is crucial to the sustainability of marine fish populations (Houde, 1987). Slight changes in mortality during early life stages can have large effects on subsequent recruitment to the fishery (Sissenwine, 1984; Houde, 1987; Juanes, 2007). A better understanding of the recruitment process can greatly benefit management of exploited stocks by enabling prediction of future harvests (Wooster and Bailey, 1987). Often, fishery-independent monitoring of juveniles is used as an index of future recruitment (Hanisko et al., 2007; Ingram et al., 2007).

A fishery-independent index of abundance is often a more reliable proxy for abundance compared to fishery-dependent data. Fishery-independent surveys are usually conducted with consistent methodologies and protocols. This consistency attempts to eliminate sources of variability and bias. One of the main sources of bias with fishery-dependent landings data are changes in catchability; this is especially true in reef fisheries where improvements in electronics have improved fishers' ability to return to productive fishing locations or where regulations have limited landings data.

Gag grouper (*Mycteroperca microlepis*) are an economically important reef fish on the continental shelf of the SE US. They are exploited both by recreational and commercial fishers, mainly using hook-and-line methods (Bacheler and Buckel, 2004). Gag grouper are

North Carolina's most valuable commercial grouper fishery, with an ex-vessel value of over $650,000 in 2007. In the North Carolina recreational fishery, gag are caught more often than any other grouper species; anglers averaged 39,660 kgs per year during the past decade (Landings data from North Carolina Division of Marine Fisheries (NCDMF) web page, 2009). Previously designated as overfished, the stock had recently (stock assessment from 2001) been designated as recovered as a result of gear restrictions, spawning season closures, decreased bag limits, and increased size limits (NCDMF). However, the most recent stock assessment completed in 2007 showed a decline in abundance due to excessively high fishing mortality rates (SEDAR 10, 2007). If current fishing mortality rates continue, gag grouper will likely move back to the overfished status. In reaction to the declining trend, a 50% reduction in harvest was proposed for 2008 (SAFMC, 2007).

The latest gag grouper assessment has been questioned because of its reliance on fishery-dependent data (see Figure 1.1 for example of conflicting data sources). Currently, there are no fishery-independent data sets (e.g. index of juvenile abundance) used in the stock assessments of gag grouper in the U.S. south Atlantic. A fishery-independent index of recruits or spawning stock biomass would likely reduce uncertainty in the assessment. The NCDMF and South Atlantic Fisheries Management Council (SAFMC) currently list a juvenile abundance index as a data and research need for gag grouper. The lack of information for this stage is due to the fact that juvenile gag grouper are relatively rare, cryptic, and inhabit areas that are difficult to sample with most conventional gear types (Ross and Moser, 1995).

Several studies have examined the early life history stages of gag grouper in the southeast US. In the Gulf of Mexico, Koenig et al. (1998) captured juvenile gag grouper using small trawls in seagrass beds and estimated absolute abundance using mark-recapture techniques. Ross and Moser (1995) used seine nets to capture juvenile gag grouper settled in seagrass beds in North Carolina. Other studies have followed these methods to capture juvenile gag (Johnson and Koenig, 2005; Fitzhugh et al., 2005; Renan et al., 2006; Casey et al., 2007) but most of these studies were conducted in the Gulf of Mexico. In the US south Atlantic, studies on gag were conducted by Keener et al. (1988) and Rutten (1998). Keener et al. (1988) collected post-larval gag in South Carolina using neuston nets during flood tides. Rutten (1998) collected emigrating gag from New River, NC using channel nets (a passive gear that targets shrimp).

Here, I assess the potential for developing an index of abundance for gag grouper in North Carolina (NC). First, I examined factors that influence the timing of post-larval gag grouper ingress through Beaufort Inlet, NC and the feasibility of creating an index of post-larval abundance from historical bridgenet samples. Second, I examined factors that explain variability in juvenile gag grouper catch-per-unit-effort (CPUE) in NC seagrass beds to help refine any future juvenile gag grouper sampling. Lastly, the utility of the annual post-larval index was tested through comparisons to spawning stock biomass and age-1 abundance. The ultimate goal was to determine which life stage and sampling gear are most appropriate to index gag grouper abundance.

1.2 Materials and Methods

1.2a *Life History*

Gag grouper exhibit life history characteristics that are typical of a K-selected species; slow growth, large size, late reproduction, and long life spans (Parrish, 1987). Gag are protogynous hermaphrodites, starting their life as females (sexually mature at 4-5 years at length of approximately 24-26 inches) then transforming into males between 8 and 10 years of age (32-40 inches) (Collins et al., 1987; NCDMF). Gag recruit to the commercial and recreational fisheries at approximately four years old (24-26 inches); possibly allowing them to spawn once as females before being harvested. Spawning aggregations form offshore on the US south Atlantic coast of the United States in late winter-early spring, with post-larval ingress into South Carolina estuaries peaking in early May (Keener et al.,1988). Predation mortality on post-larvae and juveniles has been hypothesized to be low during their estuarine residence because of their relatively large size (Koenig and Coleman, 1998). In NC, gag begin to move from seagrass beds to more complex substrates (oyster reef, dock pilings, rock jetties) within the estuary between late June and July (Ross and Moser, 1995). Massive emigration from the estuary to nearshore ocean hard bottom habitats occurs in autumn in conjunction with a drop in water temperature brought on by the passage of cold fronts (Ross and Moser, 1995).

1.2b *Collection of post-larval gag grouper*

Ichthyoplankton sampling from the Pivers Island bridge (bridgenet sampling) has been conducted since 1986 by the NOAA Beaufort, NC laboratory (Figure 1.2). Pivers Island is located in Back Sound, approximately 1.5 kilometers from Beaufort Inlet. From 1986 to 2008, ichthyoplankton were sampled from ~November to April/May using a 1x2m neuston net with 1mm mesh with the following exceptions. Sampling occurred throughout the year beginning in 2005. In 2007, a new bridge was constructed and the old bridge was destroyed. During this transitional phase (from ~March, 2007 to March, 2008), a 1m diameter ring net with 500 um was used for weekly sampling. For both nets, four consecutive surface tows were conducted each week; volume of water filtered for each tow was determined using a flowmeter with the goal of filtering approximately $100m^3$ of water per tow. Sampling was conducted on nighttime flood tides, approximately two and a half hours before high tide as predicted by NOAA (http://tidesandcurrents.noaa.gov). Gag grouper caught in the bridgenet (those that are planktonic) will be referred to in this document as post-larvae after Keener et al. (1988).

Because historic weekly sampling caught post-larval gag grouper infrequently, nightly sampling was done in spring of 2007 and 2008 to better define the ingress period and factors related to ingress. In 2007, nightly sampling began 2 May and continued through 25 June. Given zero catches of gag grouper in nightly samples in June 2007 and weekly samples in June 2005 and 2006 (see Table 1.1), nightly sampling was moved one month earlier in 2008; nightly sampling in 2008 began 2 April and continued through 29 May.

Nightly sampling in 2007 and 2008 was conducted with a 1m diameter (500 µm mesh) ring net and the methodologies followed exactly with that of weekly sampling described above.

Bridgenet data from 1986 to 2008 were analyzed to determine timing and magnitude of ingress of gag grouper into Beaufort Inlet, NC. Post-larval gag grouper densities (number per 1000m^3 of water filtered) were determined for each tow. Mean nightly densities were calculated by averaging the larval concentrations from the four tows conducted on a given night. Mean weekly densities were calculated for 1986-2008 by pooling all years and averaging the mean nightly densities for each respective week. Weeks within each month were blocked as follows: days 1-7 = week 1, days 8-14 = week 2, days 15-21 = week 3, days 22-28 = week 4, days 29-31 = week 5.

1.2c *Collection of juvenile gag grouper*

Collections of juvenile gag grouper were made with a 5m otter trawl (12.7 mm bar mesh in body; 3.2mm bar mesh in bag) in seagrass beds in Bogue, Back and Core sounds (Figure 1.2). Sampling was limited to seagrass beds because earlier studies have shown that juvenile gag grouper use structured habitat almost exclusively soon after settlement (Koenig and Coleman, 1998; Fitzhugh et al., 2005; Renan et al., 2006). Sampling began in mid-June and continued through mid-September in both 2007 and 2008 (some experimental sampling was done in early June during which few gag grouper were caught). Fifteen to twenty different random locations were sampled every two weeks. Random locations were generated using Hawth Tools (Beyer, 2004) within a seagrass spatial layer (Scott Chappel, NCDMF) in ArcGIS. Due to the shallowness of the seagrass beds (0.5-1.5m) all trawls were conducted within ± 1.5 h of high tide. Trawl speeds averaged 3.5km/h and were five minutes in duration; thus, trawl tracks were ~300 m long. At each trawl location, temperature, dissolved oxygen, and salinity measurements were recorded.

Seagrass assessments were also conducted at each trawl location. These assessments consisted of identification of species and determining percent coverage. The two predominant species of seagrass present in North Carolina estuaries are eel grass (*Zostera marina*) and shoal grass (*Halodule wrightii*). Percent coverage was determined using the Braun-Blanquet selection criteria where 0 = none 1 = 1-5%, 2 = 6-25%, 3 = 26-50%, 4 = 51-75%, 5 = 76-100% (Wilzbach et al., 2000). In 2007, seagrass assessments were made by a snorkeler determining coverage in a single quadrat haphazardly thrown into the trawl transect; after the first several trawl stations, it was determined that a single quadrat was not adequate to assess average seagrass coverage over the entire ~300m trawl track. Because time for more quadrats was limited due to high tide restrictions, visual estimates of percent coverage were made topside over the entire trawl track; this was feasible because of the shallow water. In 2008, percent coverage of sea grass was again made from topside visual estimates over the entire trawl tract. To determine the efficacy of this topside approach, comparisons were made to a ten-quadrat approach. The ten-quadrat approach consisted of haphazardly throwing quadrats every 30m throughout the trawl track with snorkeler assessment of seagrass coverage. The mean of these ten quadrat scores was taken as the seagrass coverage for an individual trawl. Since there was a significant positive relationship between the topside visual assessment and the ten quadrat approach ($r^2 = 0.67$, $p < 0.001$, n = 54), topside visual assessments of seagrass coverage were used in all further analyses. Since topside visual assessments were not made for the first set of random samples in 2007, these 15 tows were excluded from analyses investigating factors that influence juvenile gag catch.

The size (total length, mm) of juvenile gag grouper were plotted against date of collection**.** Growth rates were estimated during the summer months (June – Sept) using linear regression analyses. Post-larval and autumn caught juveniles were not included in this analysis because growth was limited during these times (Figure 1.8A & B). We attempted to capture juvenile gag in a variety of other gears including beam trawls, minnow traps, and seine nets. Gag grouper collected in these gears were used for growth rate analyses and in Chapter 2 only.

Prior studies have sampled egressing gag grouper juveniles from the catch of channel nets set by commercial fishers (Rutten 1998). A channel net is a fixed net (passive gear); the outgoing tidal current carries the targeted catch (shrimp) and other tidally outmigrating animals, including juvenile fishes, into the net. Sampling duration encompassed the nocturnal period of ebbing tide and ranged from 3 to 6 hours each night. In 2007, egressing juvenile gag grouper were collected in channel nets in collaboration with commercial shrimp fishermen. Channel net samples were collected in Back Sound and New River (Figure 1.2). Attempts were made to attend channel net trips in 2008 but no commercial fishers responded to my request; this was partly due to reduced local channel net shrimp catch in 2008.

I compared the positive catch rates (efforts with zero catch were excluded) for three different gear types: ichthyoplankton net, otter trawl, and channel net. Positive catch data were used because those were the only data available from channel net fishers. The gear that had the highest catch per hour was assumed to be the most efficient at collecting early life history stages of gag grouper. A bridgenet effort was equal to one hour of time; this effort consisted of four 10 minute tows, plus 20 minutes for setup, sorting, and takedown. Thirty minutes was defined as one unit of effort for the otter trawl; 5 minute tow, plus 25 minutes for sorting. The fishing time for a single channel net set is often irregular (as fishing times are dependent upon the amount of shrimp being caught on a given night) so the total fishing time on a night of channel netting was taken as the unit of effort; this averaged around 3 hours in 2007. This channel net time was also applied to an earlier data set from Ross and Moser (1995).

1.2d ***Developing an index of abundance***

Index values produced by year are of interest for developing a relative abundance index over time. In order to determine the factors driving ingress, a delta generalized linear modeling (dglm) approach (Dick, 2004) was used to fit several models to concentration of gag grouper post-larvae from 1986 to 2008 using April and May catch data. The dglm method is a combination of two generalized linear models: a binomial model which describes the variability in the proportion of positive catches (presence/absence) and a lognormal model which describes the variability in the positive catch data. We included only years with at least two positive data points. As a result, 13 out of the 23 bridgenet sampling years were included in this analysis. Modeling was done using dglm index code (Program R) provided by the NOAA-Beaufort Population Dynamics Branch. Multiple independent variables were examined including year, day, predicted peak tidal heights, sinθ, sin2θ, cosθ, cos2θ where θ = lunar day in radians. Models using biologically plausible combinations of these variables were fitted to the 1986 to 2008 post-larval ingress data. Akaike's Information Criterion (AIC) values were produced for both the binomial and the lognormal models (Burnham and Anderson, 2002); these were added together to produce a total AIC score. Models were ranked from lowest to highest AIC score. The dglm index was compared to an arithmetic index that was created by averaging the concentrations (no. per $1000m^3$) of post-larval gag grouper caught in all nights of sampling in April and May in each year.

Lunar days were defined as days from full moon and were based on a 29 day lunar cycle (full moon = 0, new moon = 14.5). The sine and cosine terms are periodic regression terms used to model lunar (single peak per month) and semi-lunar (two peaks per month) peaks in ingress (deBruyn and Meeuwig, 2001). The cosine term describes peaks at either new or full moons, the cosine 2θ term describes peaks at both new and full moons. The sine term describes peaks at either the first and third quarter of a lunar cycle, while sine 2θ describes peaks at both the first and third quarters. Models that include both a θ and 2θ term can have two peaks per lunar cycle that are unequal in amplitude (deBruyn and Meeuwig, 2001).

For a subset of years (1996-2008) observed tide heights were available from NOAA's NDBC Station BFTN7 which is located on Pivers Island. Observed tide heights were used to calculate a tide anomaly value (observed tide – predicted tide) which can be used as a proxy for wind, river discharge, and any other factors that may cause variation in local tide heights (Ogburn et al, in press). Tide anomaly and the multiple independent variables listed above were used to model the 1996 to 2008 post-larval ingress data. Biologically plausible combinations of these variables were modeled and their total AIC values were compared.

The dglm approach was also used to assess the factors that influence catch of juvenile gag grouper in seagrass beds. The dependent variable was number of gag grouper caught per tow. The independent variables investigated were year, day, Braun-Blanquet score, species of seagrass, sound, distance from Beaufort Inlet, and water temperature. AIC was used for model selection (Burnham and Anderson, 2002).

1.2e *Correlations with other indices of abundance*

The index of post-larval gag grouper abundance was compared to other indices of abundance. I tested the correlations between the dglm adjusted post-larval index with: a juvenile abundance index from Florida (need citation for this data source) and the most recent stock assessment (SEDAR 10, 2007) derived estimates of spawning stock biomass (SSB) and age-1 gag grouper abundance for the U.S. south Atlantic. The Florida Wildlife Resources Commission developed an index of abundance using beach and purse seines. Years that overlapped with my study were 1996 to 2004; four years were available for comparison. The estimates of SSB and age-1 (they are listed as age-0 in the assessment but are 9 month old fish at the start of the assessment time step, Kyle Shertzer, NOAA Beaufort, pers. comm.) gag grouper biomass are from the Southeast Data and Assessment Review (SEDAR10, 2007). The correlation between SSB in year t and age-1 fish in year t+1 was tested; SSB was also compared to the dglm post-larval index within the same year. Lastly, the dglm post-larval index from year t was compared to age-1 gag grouper estimates from year t+1.

1.3 Results

1.3a *Post-larval gag grouper*

From 1986 to 2008, post-larval gag grouper were collected at Beaufort Inlet, NC from March through May (Figure 1.3A). However, ingress in March can be considered negligible because it represents a total of only three gag grouper over a 23-year period. The majority of ingress occurred in April and May with highest catches occurring from the second week in April through the fourth week in May. Overall, post-larval gag grouper were rare with the highest weekly mean density (averaged over all years) peaking at ~eight post-larval gag per $1000m^3$ (Figure 1.3A); within a year, most positive catches were less than ~ten post-larval gag grouper per $1000m^3$ (Tables 1.1 & 1.2; Figure 1.3B & C).

The nightly sampling in 2007 and 2008 better defined the timing of ingress within a month. A total of 26 post-larval gag grouper were caught over 43 near consecutive nights in 2007 (Table 1.2). In 2007, ingressing post-larval gag grouper were only caught in May, with 88% of the annual total being caught during a seven day period (14 to 21 May; Table 1.2, Figure 1.3B). A total of 15 post-larval gag grouper were caught in 2008 during 56 consecutive nights of sampling in April and May. In 2008, there were two pulses of gag grouper ingress, one small pulse in mid-April (n=5) and a slightly larger pulse in early May (n=9); both pulses were roughly one week in duration (Table 1.2, Figure 1.3C).

Although ingress of post-larval gag grouper occurred throughout the lunar cycle, most positive observations as well as the highest concentrations occurred around new moons in April and May (Figure 1.4). The two models that provided the best fits to the ingress data contained a periodic regression term (cos Θ) indicative of a peak in ingress around the new moon time period (lunar day 14; Table 1.3A, Figure 1.4). These models had equal support and also included year and day effects. Although Model 1a contained a sin2Θ term, this term had little effect on the shape of the model due to a small coefficient (sin2Θ = 0.096, cosΘ = 0.602; Figure 1.4).

Tide anomaly data were available for 1996 to 2008. Using this reduced data set, I found no support for tide anomaly as a predictor of ingress of post-larval gag grouper (Table 1.3B). The two models that had the most support for the entire data set also had the most support in this reduced data set (models 1b and 2b; Table 1.3B).

The annual abundance of post-larval gag grouper captured at the Pivers Island bridge is highly variable (Figure 1.5). For models 1a and 2a, the dglm adjusted indices of abundance and the arithmetic mean index of abundance followed a similar trend (Figure 1.5). In many of the years prior to 2005 (when year round sampling began), the dglm indices were adjusted upward of the arithmetic index; this is likely due to reduced sampling in May for those years (Table 1.1; Figure 1.5).

1.3b *Juvenile gag grouper*

A total of 56 juvenile gag grouper were caught in 84 trawls in 2007. In 2008, 90 trawls caught 30 juvenile gag grouper. Year, day, temperature, percent seagrass coverage, seagrass species, and sound (Bogue, Back, Core) were all found to be important predictors of juvenile gag grouper catch in seagrass beds; the top three models that contained various combinations of these terms all had essentially equal support (ΔAIC<2; Table 1.4). CPUE was higher in 2007 than in 2008 and within year catch often followed the seasonal temperature trend (Figure 1.6A and B). Additionally, CPUE of juvenile gag grouper increased with increasing seagrass coverage (Figure 1.7A) and was higher in *Zostera marina* compared to *Halodule wrightii* seagrass (Figure 1.7B). Core sound had low CPUE in both years with Back sound consistently higher than Core (Figure 1.7C).

Rapid growth of juvenile gag grouper began in June and continued through September (Figure 1.8). This coincides with times of warm temperatures inside the estuary (Figure 1.6B; 2007 mean = 27.8^{o}C; 2008 mean = 27.8^{o}C). Summertime growth rates averaged 1.6mm per day in 2007 and 1.5mm per day in 2008 and were not significantly different between years (homogeneity of slopes, p = 0.86). Additionally, there was little variation among individuals within years (2007, r^2 = 0.88; 2008, r^2 = 0.70); these size data provide strong support for a single cohort.

Channel net efforts caught 6 and 51 gag grouper from New River on 10/21/2007 and 11/3/2007, respectively (this study), 45 gag grouper from Back Sound on 10/2/2007 (this study), and 267 gag grouper from Bogue Sound on 10/1/1980 – 10/2/1980 (Ross and Moser, 1995). For positive catches, the yearly catch rates of gag grouper in channel nets were an order of magnitude higher (~11 to 46 gag/hour) when compared to trawls and ichthyoplankton nets (Table 1.5). For positive tows, the otter trawl caught ~3 to 4 gag per hour and the bridgenet caught ~1 to 3 gag per hour.

1.3c *Correlations with other indices of abundance*

There was no relationship between the dglm post-larval index (model 1a) and the juvenile seine index from Florida ($r = -0.17$, $p = 0.82$). There was no correlation between SSB and age-1 gag grouper abundance estimates from the SEDAR 10 stock assessment ($r = -0.56$, $p = 0.12$; Figure 1.9A). However, there was a significant positive relationship between SSB and the dglm post-larval index ($r = 0.72$, $p = 0.03$; Figure 1.9B). No correlation was found between the dglm post-larval index from year t and the age-1 biomass estimates from year t+1 ($r = -0.05$, $p = 0.89$; Figure 1.9C).

1.4 Discussion

Analyses larval and juvenile catch

Peak ingress of post-larval gag grouper occurred near new moons predominantly in April and May. Post-larval settlement in other reef fishes occurs around new moons (Victor, 1986; Walsh, 1987; Wilson, 2001; Watson et al., 2002; Tzeng et al., 2003; Ben-David and Kritzer, 2005). In a study conducted at the Pivers Island bridgenet site, Tzeng et al. (2003) found that peak ingress of gray snapper occurred around new moons. Keener et al. (1988)

also found peak ingress of gag grouper on nighttime flood tides in April and May and noted that temporal variability in ingress corresponded to temporal patterns in back-calculated hatch dates; they found that most hatch dates occurred around times of full moons with a mean PLD of ~ 43 days. Based on roughly a 29 day lunar cycle, this results in most ingress around new moons. Strong tides and low ambient light around new moons might be ideal for transport into the estuary and predator avoidance.

The bridgenet program at Pivers Island was designed to target ingressing winter-spawned larvae during nighttime flood tides. Prior to 2005, the inconsistent and low annual catch of gag grouper post-larvae at the Pivers Island bridgenet was partly a result of a sampling protocol that often missed the critical late April through May gag grouper ingress period. The dglm post-larval index values are adjusted for years in which sampling did not occur during optimal days of the year or around optimal lunar days. To increase the number of gag grouper post-larvae caught at the Pivers Island bridgenet, weekly sampling should be conducted in both April and May with nightly sampling ~5 days before through 5 days after the new moon.

The similarities between this study and that of Keener et al. (1988) suggest that gag grouper post-larvae in North Carolina and South Carolina are from spawning grounds that contain adults with similar behaviors; data from both states could be combined to design a more comprehensive US south Atlantic index. One of the downfalls of the NC index is that it only includes data from a single site. Having multiple sampling sites along the U.S. south Atlantic coast would help guard against variability at a single site that is not representative of the entire stock.

Larval and juvenile abundance indices are used in fishery stock assessment. For example, Hanisko et al. (2007) found a strong correlation between catch of larval and adult red snapper from fishery-independent data collected in the SEAMAP surveys in the Gulf of Mexico; this larval index is now used in the stock assessment of Gulf of Mexico red snapper. Fitzhugh et al. (2003) found alternating strong and weak year classes in the age structure of gag grouper caught in the Gulf of Mexico; Johnson and Koenig (2005) found juveniles to have a similar pattern and they hypothesized that juvenile abundance estimates could be useful to forecast the future age structure and production of the fishery. Given the relationship that I found between gag grouper SSB and post-larval abundance, I recommend that NOAA consider the dglm post-larval index as a fishery-independent index of SSB in the next US south Atlantic gag grouper assessment. More years of data throughout a wider range of SSB estimates will allow a better assessment of the utility of the post-larval index.

Year, day of year, percent seagrass coverage, seagrass species and sound explained variability in juvenile gag grouper catch. Similarly, Casey et al. (2007) found year, month, sound, percent seagrass coverage, water depth, and location (shoreline vs. shoal) to be significant predictors of juvenile gag grouper abundance in southwest Florida estuaries. Thus, habitat use of juvenile gag grouper is similar between Gulf of Mexico and North Carolina estuaries and this information can be used to stratify existing monitoring programs if a goal was higher catches of juvenile gag grouper. Catch of juvenile gag grouper was higher in eel grass when compared to shoal grass. In a laboratory experiment, Levin and Hay

(2003) found that juvenile gag preferred eel grass over shoal grass and postulated that this was because of the relative blade densities of the two seagrass species. Although this mechanism might be responsible for the pattern observed here, the presence of each species of seagrass is also related to time of year (day) and water temperature (temp). Eel grass is a cold water species that is most abundant during early summer (June-July), while shoal grass is more prominent during late summer months (July-Sept); thus, the peak abundance of gag grouper in seagrass may correspond more closely with peak in eel grass abundance.

Temperature and day of year were important predictors of juvenile gag grouper catch but are confounded. Catch of juvenile gag grouper in seagrass beds increased throughout the summer, peaked in July or August, and declined afterwards. This trend in relative abundance was consistent with what has been found throughout the US south Atlantic (Ross and Moser, 1995) and the Gulf of Mexico (Casey et al., 2007; Koenig and Coleman, 1998; Renan et al., 2006). The decline in abundance of gag grouper in seagrass beds is thought to a result of emigration to inshore and offshore hard bottom habitats. High catches of gag grouper in channel nets in late summer and fall support this theory (Rutten, 1998; Ross and Moser, 1995; this study). Ross and Moser (1995) found that juvenile gag grouper emigration coincides with abrupt drops in water temperatures brought on by cold fronts. I observed this phenomenon in 2008 when Tropical Storm Kyle hit the study area in late August. After this storm which brought with it cold, rain, and strong winds, no gag grouper were caught in the study area. Commercial channel net fishermen in the Cape Lookout region also reported no catch of gag grouper after this storm.

Although gag grouper are generally thought to be a long-lived, slow growing species, they grow extremely fast during their first year. The lengths of all ingressing gag grouper showed little variation despite variable ingress dates (mean size = 19 mm TL ± 1.59 SD). Upon settlement, gag grew rapidly during summer months. Growth slowed in fall presumably due to drops in water temperature. Another possible explanation for the slowing of growth rates later in the summer could be the emigration of the larger, faster growing individuals out of seagrass beds and sounds; this would leave smaller or slower growing gag left for capture. There was low variability in size during summer months. The pulsed post-larval ingress period and the low variability in size over time suggests that gag grouper recruit to North Carolina as a single cohort. This is consistent with what has been described earlier in the Carolinas (Keener et al., 1988; Ross and Moser, 1995) and in the Gulf of Mexico (Koenig and Coleman, 1998; Renan et al., 2006). However, there was large variability in size of gag grouper caught in channel nets during fall months in 2007. This variability in size could be because channel nets sample gag grouper that resided in other habitats that could cause differences in growth rates, (e.g. oyster reefs and other hard bottom substrates) or that the channel net collected fish were from a different estuary than the summer trawl caught fish. Future investigations into growth rates and eventual size at egress by habitat, in concert with tagging emigrating gag, could provide insight into contributions of each habitat type to adult populations.

Gear comparisons

Channel nets proved to be the most productive and efficient (highest positive catches per hour) gear for sampling the early life history stages of gag grouper. Additionally, channel nets may eliminate the bias associated with other gear types. One limitation of trying to sample inside the estuary is that in addition to seagrass, juvenile gag use oyster reef and other hard bottom habitat as they grow (Ross and Moser, 1995). Conventional sampling gear types (i.e. trawls) are difficult to use in these habitats. Ross and Moser (1995) were not able to collect samples from hard bottom substrates within the estuary but did attempt to qualitatively observe the numbers of juvenile gag grouper around rock jetties using SCUBA. In this study, minnow traps set near hard substrate were inefficient at capturing juvenile grouper. Sampling egressing juveniles using channel nets negates the problems associated with sampling via bridgenet (i.e., limited spatial coverage, extremely rare in ichthyoplankton) or trawling in seagrass beds (i.e., sampling only a portion of known juvenile habitat, rare in trawl collections). Since gag grouper caught in channel nets are assumed to be emigrating to offshore reefs, they have survived the life stages where most mortality is known to occur. Rutten (1998) also made a similar argument for indexing juvenile gag grouper with channel nets; over a three year period (1990-1992), he collected 2,518 emigrating juvenile gag in channel nets fished from August through October in New River, NC. One obstacle to overcome with using this gear as a monitoring tool is that sites where channel netting is most effective are used by commercial channel net fishers.

Ichthyoplankton sampling and otter trawling were an order of magnitude less productive when compared to channel nets. Howevever, bridgenetting for ichthyoplankton may prove to be an important fishery-independent sampling tool for reasons described below. The bridgenet program has recently switched to year-round weekly samples; additional nightly sampling around new moons in May could result in more precise annual estimates of concentration. Additionally, ichthyoplankton sampling for gag grouper has begun in South Carolina and Georgia (M. Reichert, SCDNR, pers. comm.; C. Belcher GADNR, pers. comm.) which will ensure a US south Atlantic index that is comprehensive and informative. For juvenile sampling, the refinement of an existing bottom trawl survey might be more appealing to monitoring agencies when compared to designing a new survey using the non-familiar channel-net gear.

Regardless of catch rate, all gears showed a similar trend in relative abundance between 2007 and 2008. Catches of gag grouper were higher in 2007 than in 2008 in the bridgenet and otter trawls. Although no gag grouper were caught in 2008 in this study, anecdotal reports from commercial fishermen stated that catches were lower than in 2007.

Management implications

There is much interest in developing a fishery-independent index for gag grouper in the US south Atlantic. The SSB and age-1 abundance estimates from SEDAR10 were not related implying that factors acting between egg production and the age-1 life stage obscure effects of SSB. The significant positive relationship between SSB and the dglm post-larval index is encouraging; the dglm index of post-larval gag grouper may prove to be a reliable indicator of SSB. I recommend that sampling for post-larval gag grouper be refined in the NOAA bridgenet sampling and the dglm index be considered for use in the next stock assessment for US south Atlantic gag grouper.

The post-larval index created in this study does not appear to be a good predictor of future stock strength. It is unknown if catch of juvenile grouper in the estuary will be a good predictor of recruitment to the US south Atlantic fishery although results for the Gulf of Mexico are encouraging (Johnson and Koenig, 2005). I recommend juvenile gag grouper be collected with channel nets throughout the US south Atlantic. Costs for such a monitoring program and the logistical constraints will have to be overcome for this to become a reality. Individual states might also consider adjusting their current monitoring programs to improve their ability to catch juvenile gag grouper. For example, the NC Division of Marine Fisheries plans to use these results when they begin a seagrass trawling program in summer 2009.

Table 1.1. Weekly concentrations (no. per 1000m^3) of gag grouper post-larvae from NOAA Fisheries bridgenet sampling program at Pivers Island, NC. Data represent the mean of four tows conducted on a single night per sampling week. Double dash = no fifth week. NS = no sampling.

	March					April					May					June				
Year	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
1986	0	0	0	0	--	0	0	0	0	--	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
1987	0	0	0	0	--	0	0	0	0	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
1988	0	0	0	0	0	0	0	0	1.72	0	4.03	NS	NS	NS	NS	NS	NS	NS	NS	NS
1989	0	0	0	0	0	0	0	1.61	1.05	--	1.44	NS	NS	NS	NS	NS	NS	NS	NS	NS
1990	0	0	0	0	--	0	0	7.17	0	--	0	NS	NS	NS	NS	NS	NS	NS	NS	NS
1991	0	0	0	0	--	0	0	0.96	NS	--	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
1992	1.46	0	0	0	--	1.70	0	0	3.25	2.80	0	NS	NS	NS	NS	NS	NS	NS	NS	NS
1993	0	0	0	0	0	0	6.13	0	3.55	--	2.42	3.55	29.20	NS	NS	NS	NS	NS	NS	NS
1994	0	0	0	0	0	0	0	36.41	6.70	--	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
1995	0	0	0	0	--	0	0	0	0	--	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
1996	0	0	0	0	--	1.05	0	0	2.28	--	0	NS	NS	NS	NS	NS	NS	NS	NS	NS
1997	0	0	0	0	--	0	1.47	1.63	0	0.98	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
1998	0	0	0	0	0	0	0	8.34	0	--	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
1999	0	0	0	0	0	0	0	0	0	--	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2000	0	0	1.84	0	--	0	2.31	0	0	--	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2001	0	0	0	2.08	--	0	4.24	0	27.27	--	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2002	0	0	0	2.60	--	0	0	4.34	0	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2003	0	0	0	0	--	1.46	0	0	0	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2004	0	0	0	0	0	0	0	0	0	--	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2005	0	0	0	0	0	0	0	0	0	--	3.90	2.08	0	0	--	0	0	0	0	0
2006	0	0	0	0	0	0	0	0	6.13	--	16.17	0	2.27	0	0	0	0	0	0	0
2007	0	0	0	0	0	0	0	0	0	--	0	1.77	8.78	3.32	0	0	0	0	0	0
2008	0	0	0	0	0	0	0	0	0	0	0.67	0	0	0	--	0	0	0	0	0
Mean	0.06	0.00	0.08	0.21	0.00	0.18	0.62	2.63	2.36	0.54	2.86	1.48	8.05	0.83	0.00	0.00	0.00	0.00	0.00	0.00
Se	0.06	0.00	0.08	0.14	0.00	0.10	0.33	1.61	1.23	0.22	1.03	0.32	2.58	0.35	0.00	0.00	0.00	0.00	0.00	0.00

Table 1.2. Nightly concentrations (no. per 1000m^3) of post-larval gag grouper at the Pivers Island, NC bridgenet station. Values represent the mean of four tows on a given night. NS= no sampling.

2007				
Day	**March**	**April**	**May**	**June**
1	NS	NS	0	0
2	NS	NS	NS	0
3	NS	NS	0	0
4	NS	NS	NS	0
5	NS	0	0	0
6	NS	NS	NS	0
7	0	NS	0	0
8	NS	NS	NS	0
9	NS	NS	1.77	0
10	NS	NS	NS	0
11	NS	NS	NS	0
12	NS	0	0	0
13	NS	NS	NS	0
14	0	NS	NS	0
15	NS	NS	4.41	0
16	NS	NS	8.78	0
17	NS	NS	0	0
18	NS	NS	0	0
19	NS	0	17.62	0
20	NS	NS	3.25	0
21	0	NS	2.00	NS
22	NS	NS	0	NS
23	NS	NS	0	NS
24	NS	NS	3.32	NS
25	NS	NS	0	NS
26	NS	0	6.19	NS
27	NS	NS	0	0
28	0	NS	0	NS
29	NS	NS	0	NS
30	NS	NS	0	NS
31	NS		0	

2008				
Day	**March**	**April**	**May**	**June**
1	NS	NS	0	NS
2	NS	0	0	NS
3	NS	0	2.21	NS
4	NS	0	1.83	NS
5	NS	0	1.77	0
6	0	0	1.34	NS
7	NS	3.04	1.43	NS
8	NS	0	1.34	NS
9	NS	0	1.57	NS
10	NS	0	3.20	NS
11	NS	0	0	0
12	0	NS	NS	NS
13	NS	0	0	NS
14	NS	0.48	0	NS
15	NS	2.37	2.56	NS
16	NS	0	0	NS
17	NS	0	0	0
18	NS	0	0	NS
19	0	0	0	NS
20	NS	0	0	NS
21	NS	0	0	NS
22	NS	0	0	NS
23	NS	0	0	NS
24	NS	0	0	NS
25	NS	0	0	0
26	0	0	0	NS
27	NS	0	0	NS
28	NS	NS	NS	NS
29	NS	0	0	NS
30	NS	0	0	NS
31	NS		0	

Table 1.3. List of models fitted to gag grouper post-larval concentrations using the delta generalized linear model approach (see text for details) for (A) 1986-2008 and (B) 1996-2008. Models are ranked by total AIC scores. **Θ** = lunar day values in radians.

(A)

ID	**Model**	**AIC total**	**Δ AIC**
1a	Conc = Year + Day + Day^2 + COSΘ + SIN2Θ	431.03	0.00
2a	Conc = Year + Day + Day^2 + COSΘ	431.91	0.88
3a	Conc = Year + Day + Day^2 + Predicted tide	438.53	7.50
4a	Conc = Year + Day + Day^2 + SIN2Θ	441.60	10.57
5a	Conc = Year + Day + Day^2	442.12	11.09
6a	Conc = Year + Day + Day^2 + SINΘ	443.79	12.76
7a	Conc = Year + COSΘ + SIN2Θ	444.18	13.15
8a	Conc = Year + COSΘ	444.55	13.52
9a	Conc = Year + Day + Day^2 + COS2Θ	444.69	13.66
10a	Conc = Year + COSΘ + COS2Θ	445.09	14.06
11a	Conc = Year + Day + Day^2 + COS2Θ + SINΘ	446.43	15.40
12a	Conc = Year	452.00	20.97
13a	Conc = Year + Day	452.62	21.59

(B)

ID	**Model**	**AIC total**	**Δ AIC**
1b	Conc = Year + Day + Day^2 + COSΘ + SIN2Θ	292.63	0.00
2b	Conc = Year + Day + Day^2 + COSΘ	296.60	3.97
3b	Conc = Year + COSΘ + SIN2Θ	297.03	4.40
4b	Conc = Year + COSΘ	300.03	7.40
5b	Conc = Year + COSΘ + COS2Θ	302.01	9.38
6b	Conc = Year + Day + Day^2 + Predicted tide	303.70	11.07
7b	Conc = Year + Day + Day^2 + SINΘ	309.57	16.94
8b	Conc = year + Day + Day^2 + Observed tide	310.44	17.81
9b	Conc = Year + Day + Day^2 + SIN2Θ	311.75	19.12
10b	Conc = Year + Day + Day^2 + COS2Θ + SINΘ	312.16	19.53
11b	Conc = Year + Day + Day^2	313.31	20.68
12b	Conc = Year	315.26	22.63
13b	Conc = Year + Day + Day^2 + Tide anomaly	315.80	23.17
14b	Conc = Year + Day + Day^2 + COS2Θ	316.43	23.80
15b	Conc = Year + Day	318.72	26.09

Table 1.4. List of models fitted to catch of juvenile gag grouper from trawl sampling in seagrass beds using the delta generalized linear model approach (see text for details). BB = Braun-Blanquet coverage class, spc = species of seagrass, BI = distance from Beaufort Inlet, and sound = body of water (Bogue, Back, or Core Sound) where collected.

ID	**Model**	**AIC total**	**ΔAIC**
1c	Gag = Year + Day + Day^2 + BB + Sound	274.90	0.00
2c	Gag = Year + Day + Day^2 + BB + Sound + spc	275.44	0.54
3c	Gag = Year + BB + Sound + Temp + Temp^2	276.74	1.84
4c	Gag = Year + BB + Sound	277.06	2.16
5c	Gag = Year + BB + spc + Sound + Temp + Temp^2	278.49	3.59
6c	Gag = year + BB + spc + sound	279.62	4.72
7c	Gag = Year + Day + Day^2 + BB + Sound + Sound*Year	279.65	4.75
8c	Gag = Year + Day + Day^2 + BB + Sound + Temp + Temp^2	279.84	4.94
9c	Gag = Year + Day + Day^2 + BB + Sound + spc + BI	282.71	7.81
10c	Gag = Year + Day + Day^2 + BB	284.41	9.51
11c	Gag = Year + Day + Day^2 + BB + BI	285.32	10.42
12c	Gag = Year + BB	286.43	11.53
13c	Gag = Year + BB + Temp + Temp^2	287.19	12.29
14c	Gag = Year + Day + Day^2 + BB + Sound + spc + BI + Temp + Temp^2	287.88	12.98
15c	Gag = Year + Day + Day^2 + BB + BI + Temp + Temp^2	288.95	14.05
16c	Gag = Year + BB + spc	289.16	14.26
17c	Gag = Year + Day + Day^2 + spc	306.46	31.56
18c	Gag = Year + Day + Day^2 + Sound	310.21	35.31
19c	Gag = Year + spc + Temp + Temp^2	311.02	36.12
20c	Gag = Year + Sound + Temp + Temp^2	311.17	36.27
21c	Gag = Year + Day + Day^2	312.70	37.80
22c	Gag = Year	313.39	38.49
23c	Gag = Year + Day + Day^2 + BI	313.85	38.95
24c	Gag = Year + Day	316.17	41.27
25c	Gag = Year + BI + Temp + Temp^2	317.06	42.16

Table 1.5. Comparison of gag grouper catch per hour for bridgenet, otter trawl, and channel net. Only data from efforts with positive catches were included because those were the only data available from channel net gear. One unit of effort includes time to sort gag out of samples and was defined as follows: bridgenet = 1hr; trawl = 0.5hr; channel net = 3hrs. *Data for 1981 from Ross and Moser (1995). ND = no data.

Gear	**Year**	**# gag**	**# efforts**	**# hrs**	**Gag per hr**
Bridgenet	2007	26	10	10	2.6
	2008	15	14	14	1.07
Trawl	2007	53	25	12.5	4.24
	2008	30	20	10	3
Channel net	1981*	276	2	6	46
	2007	102	3	9	11.33
	2008	ND	ND	ND	ND

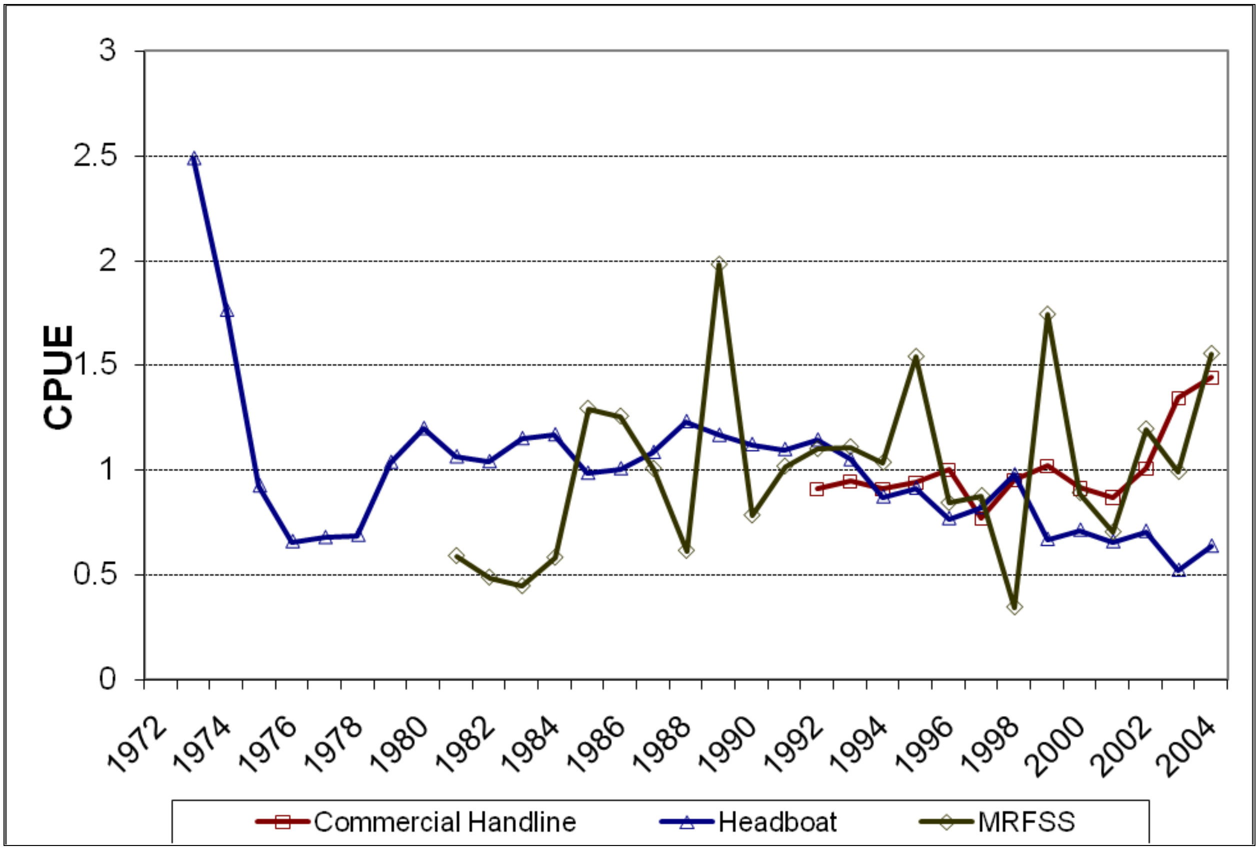

Figure 1.1. Fishery-dependent indices of abundance used in the most recent 2007 stock assessment for gag grouper (SEDAR 10, 2007).

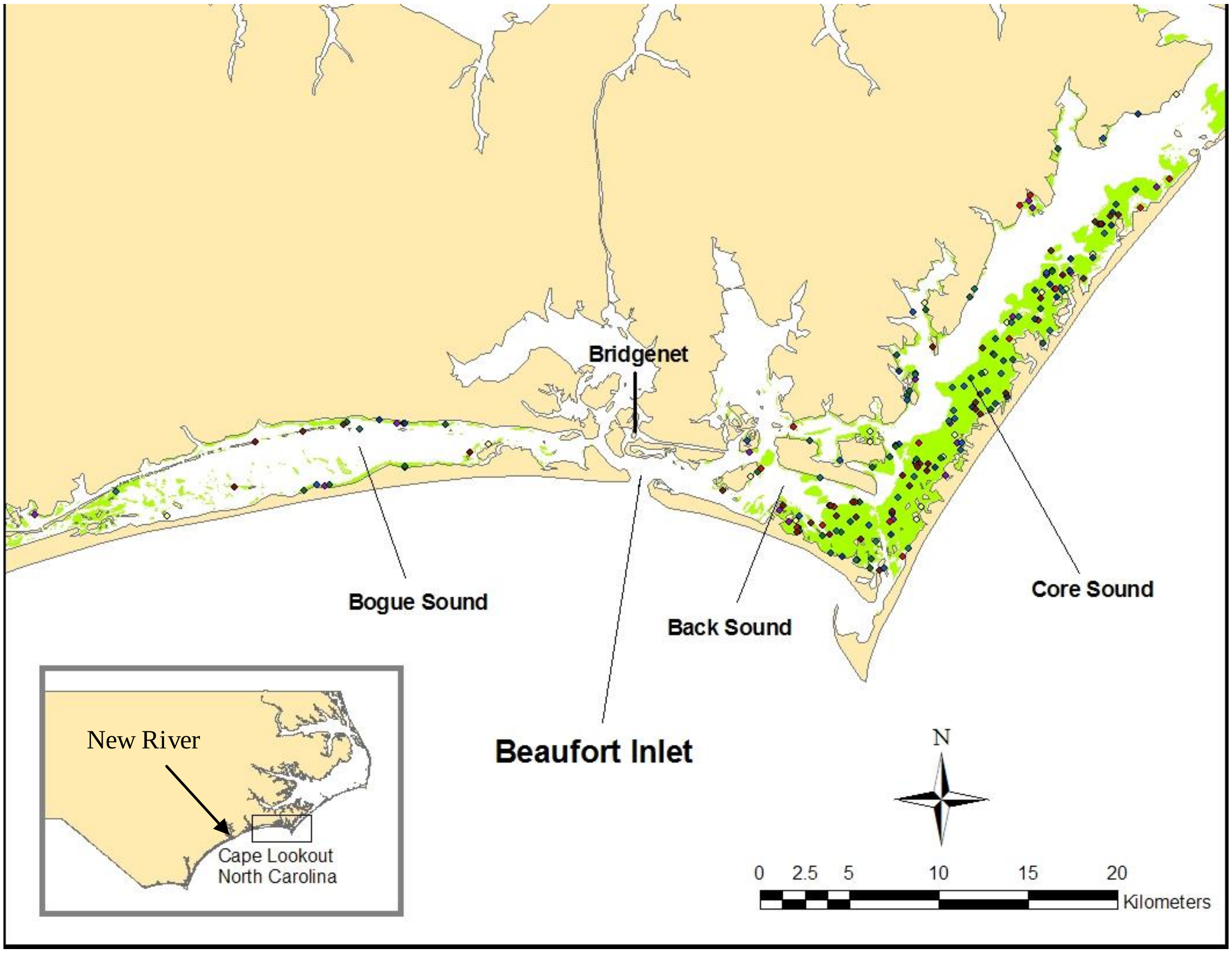

Figure 1.2. Map of the study site around Cape Lookout, NC. Bridgenet is located ~1.5km inside of Beaufort Inlet. Shaded areas inside of Bogue, Back, and Core sounds represent seagrass beds mapped by NOAA. Points inside of seagrass beds represent randomly generated stations where otter trawls were conducted in 2007 and 2008; different colors represent different sampling periods.

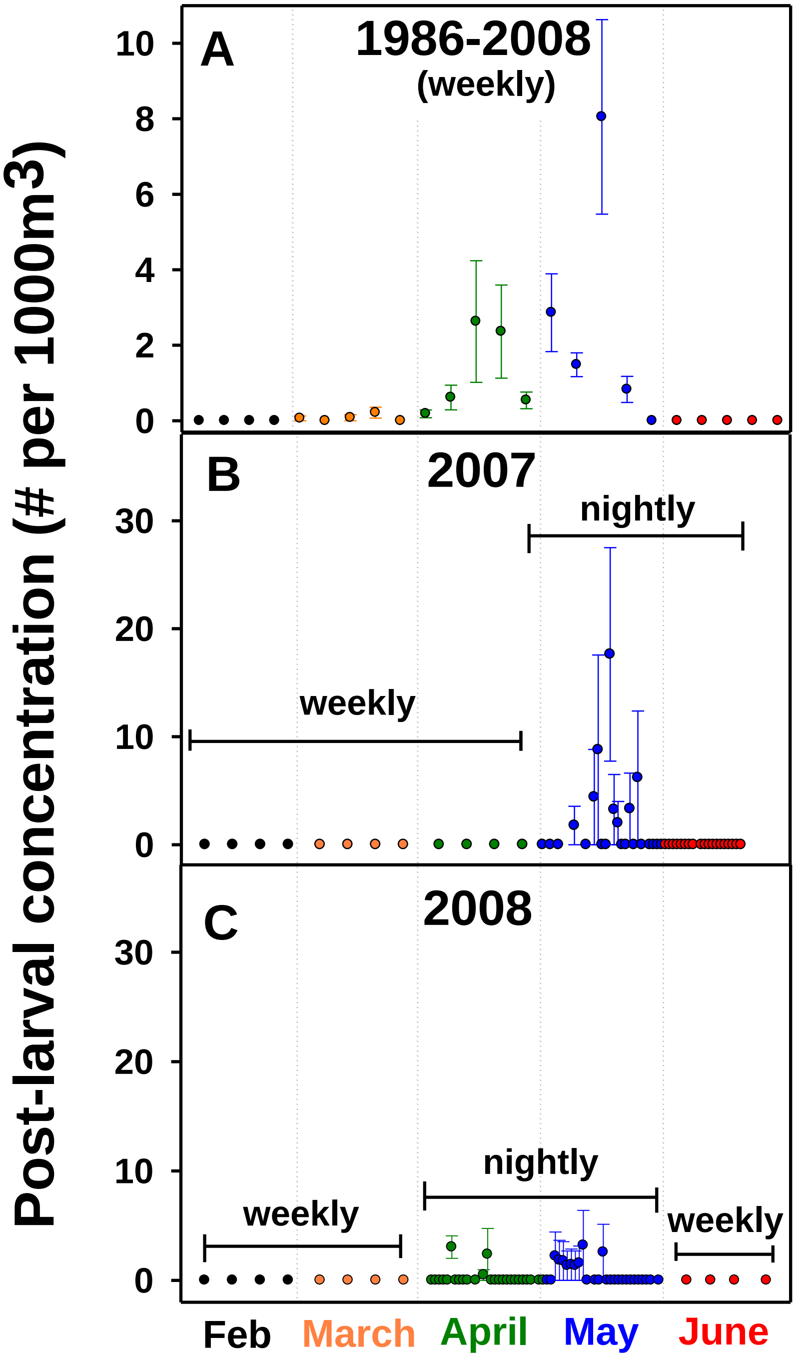

Figure 1.3. Mean post-larval gag grouper concentrations (# per 1000m^3) collected from the Pivers Island bridgenet site during February – June from (A) weekly sampling from 1986 to 2008 (see Table 1 for effort information), (B) weekly and nightly sampling in 2007, and (C) weekly and nightly sampling in 2008 (see Table 2 for effort information).

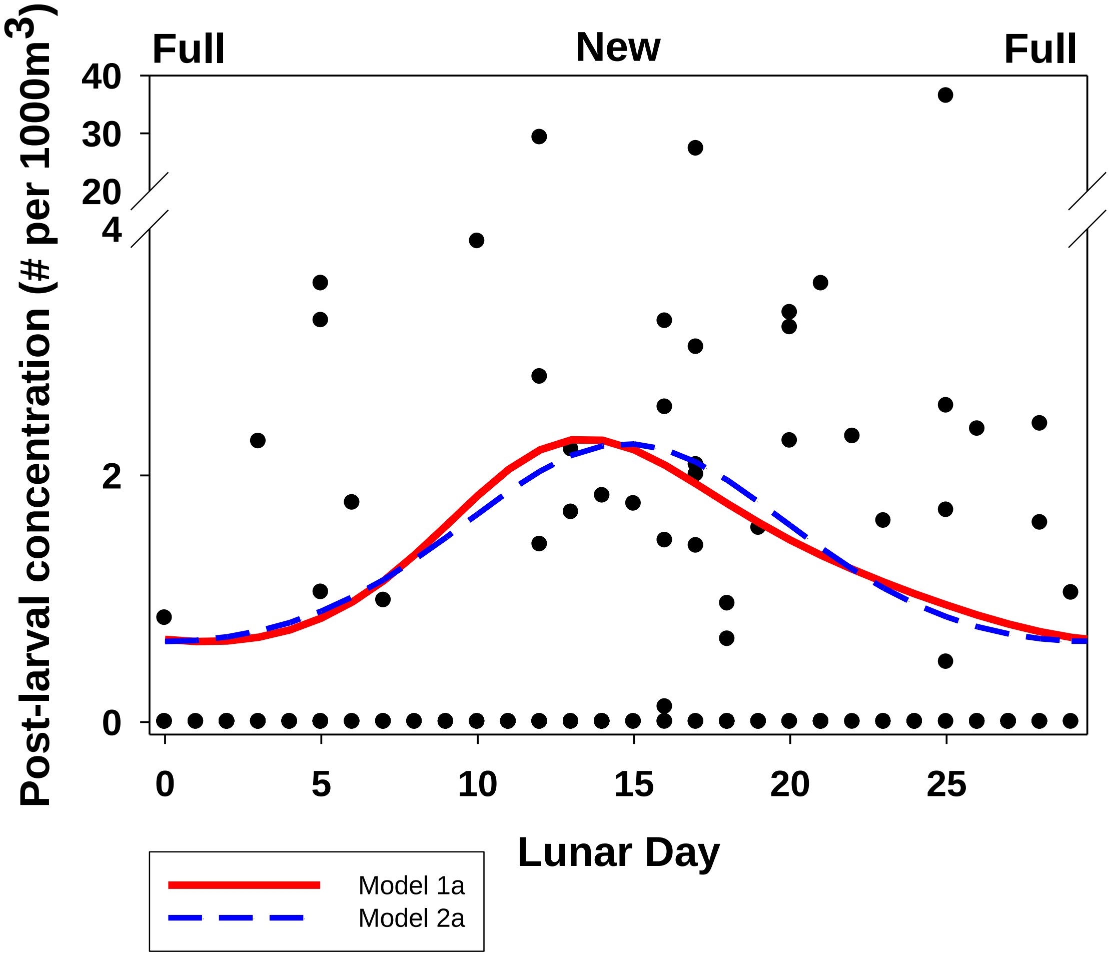

Figure 1.4. Concentration of post-larval gag grouper (# per 1000m^3) as function of lunar day. The two models with the most support [model 1a (Year + Day + Day^2 + COSΘ + SIN2Θ) and Model 2a (Year + Day + Day^2 + SINΘ) provide similar fits to the data with peak ingress occurring around the middle of the lunar cycle or the new moon periods.

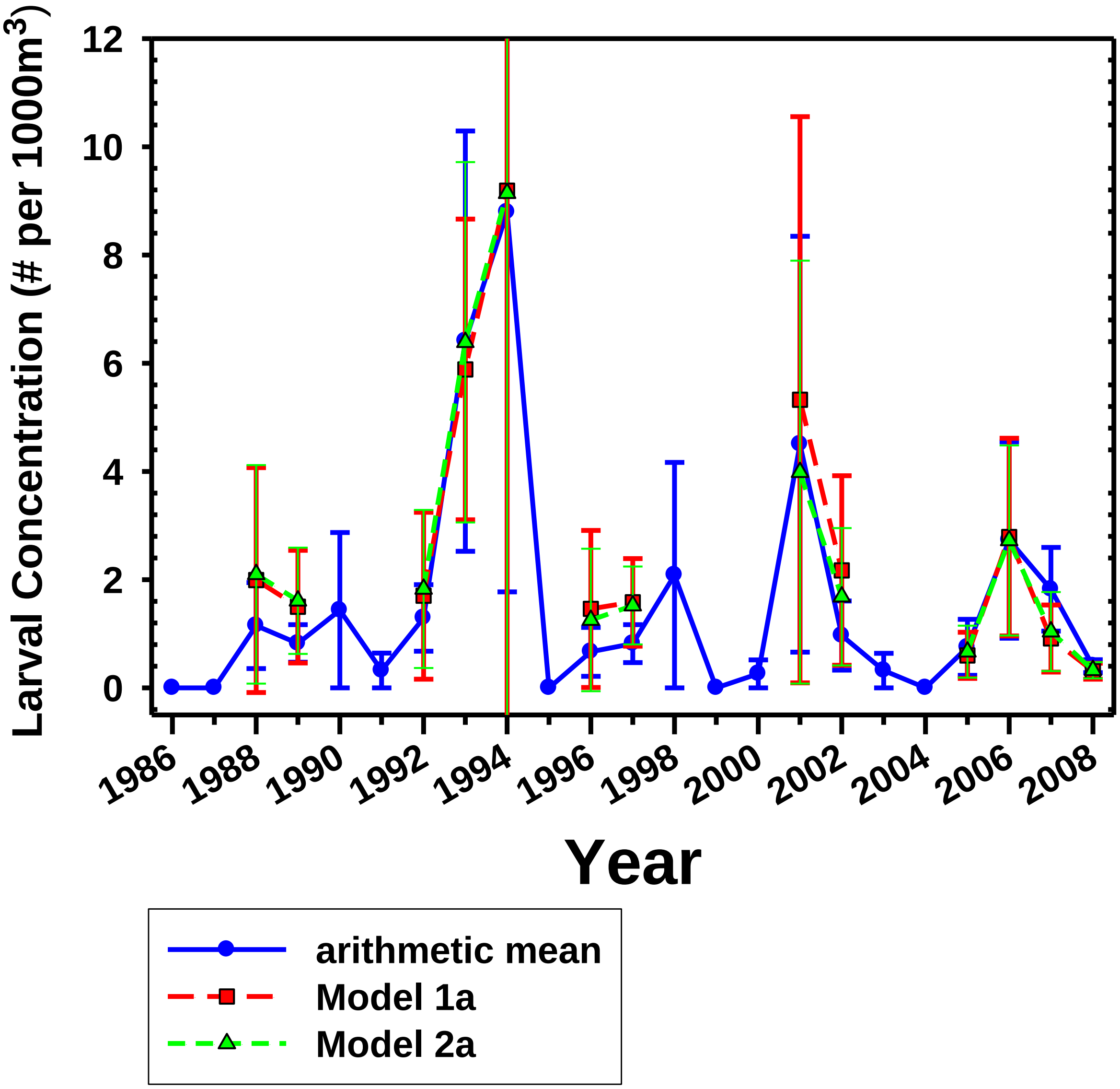

Figure 1.5. Annual post-larval gag grouper concentrations using arithmetic mean and adjusted values from the delta generalized linear model fits for models 1a (Year + Day + Day^2 + COSΘ + SIN2Θ) and 2a (Year + Day + Day^2 + COSΘ). The delta generalized modeling approach was only used for years that contained at least two days of gag grouper post-larval catch.

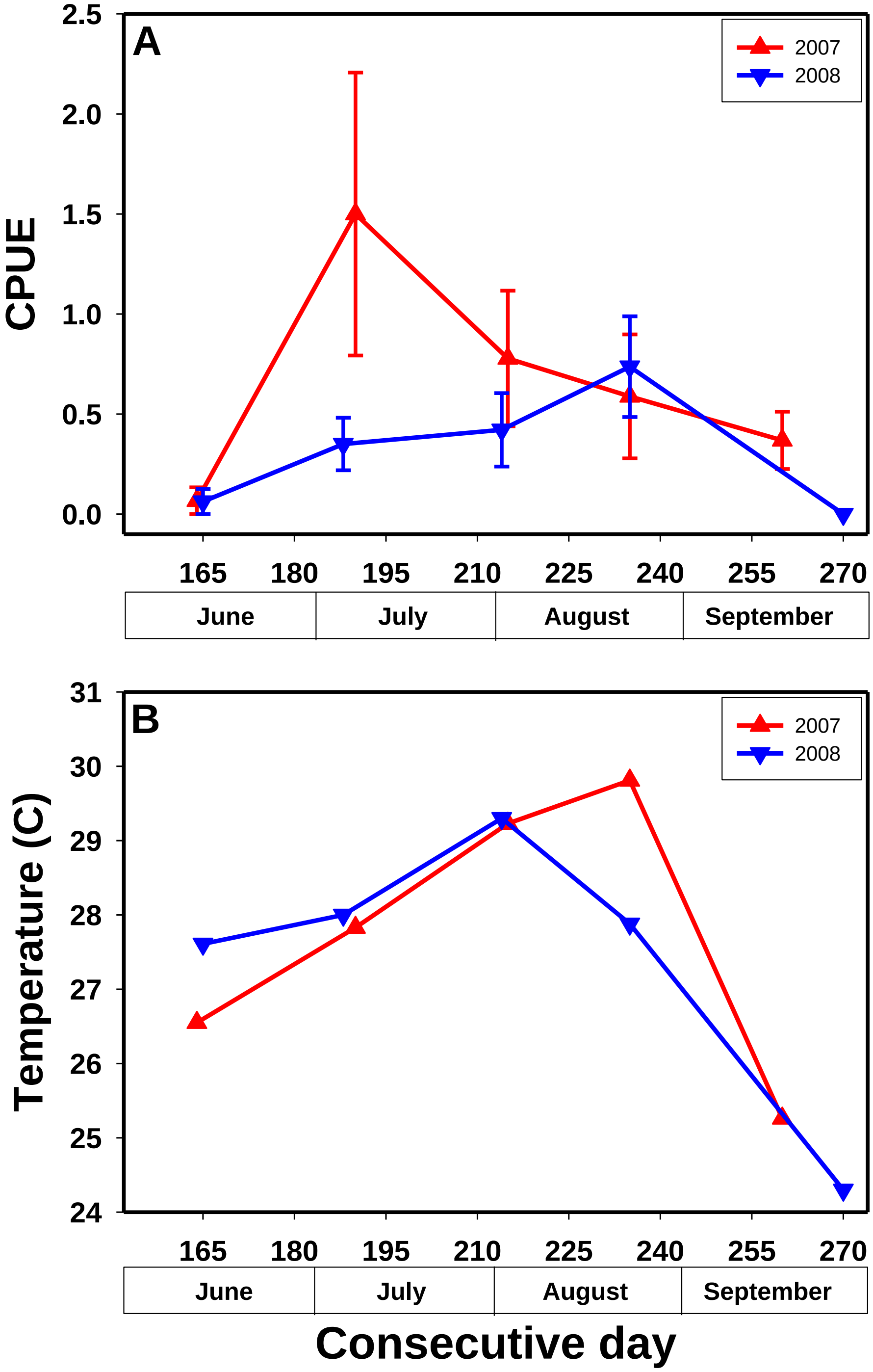

Figure 1.6. (A) CPUE of juvenile gag grouper in seagrass trawls plotted by mean date of each sampling period. (B) Temperature is plotted by mean date of each sampling period.

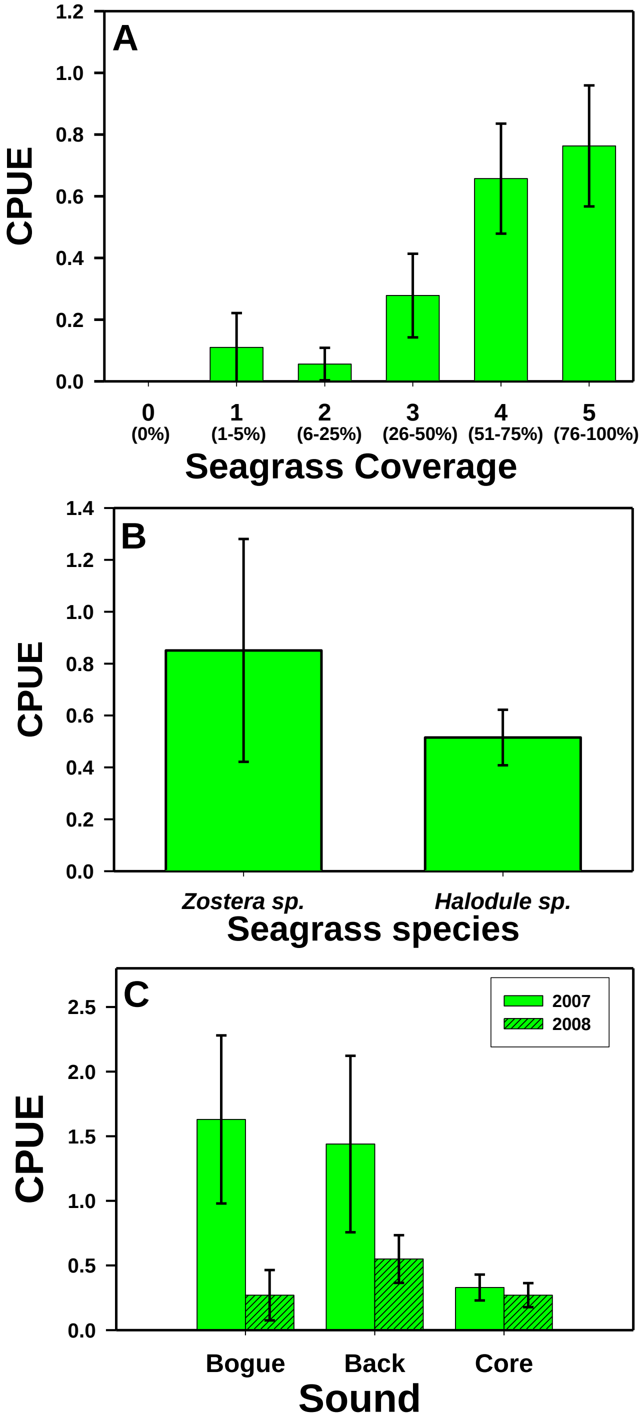

Figure 1.7. CPUE of juvenile gag grouper by (A) seagrass coverage class (Braun-Blanquet scores), (B) species of seagrass (eel grass *Zostera sp.* or shoal grass *Halodule sp.*), and (C) sound where collected.

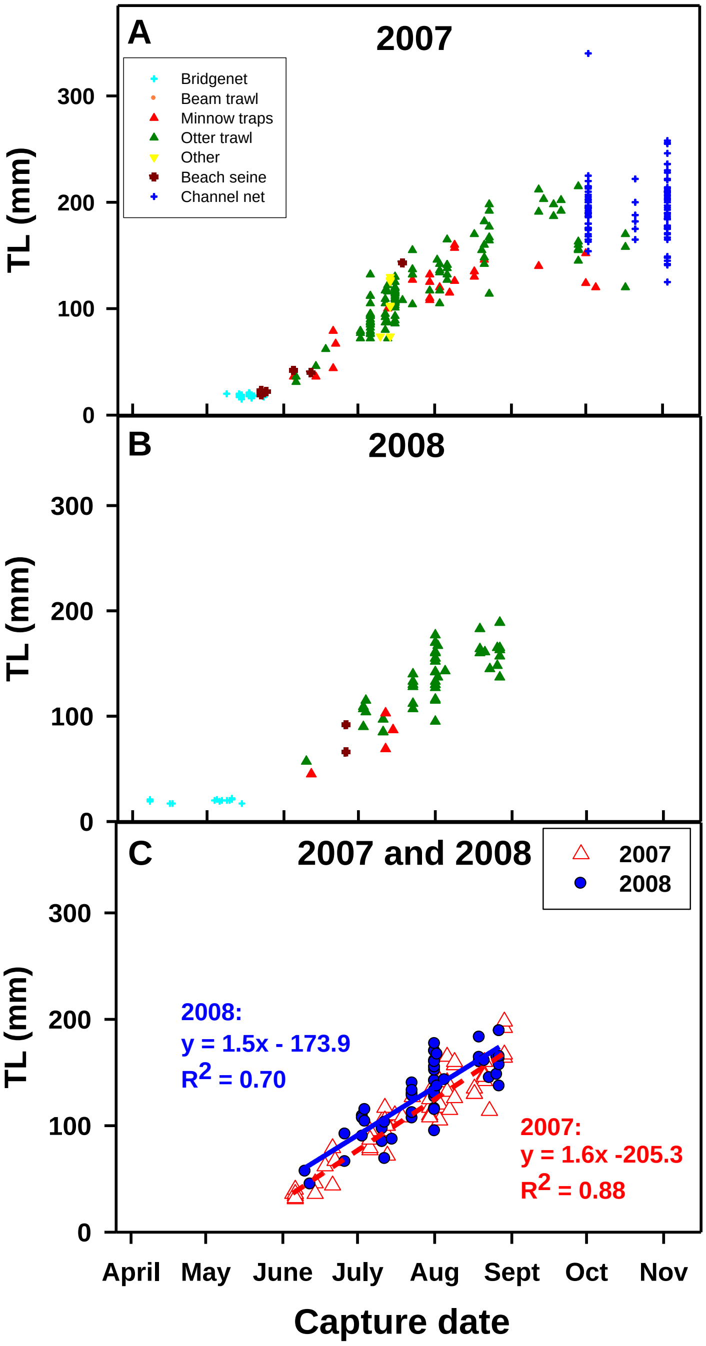

Figure 1.8. Total length of post-larval and juvenile gag grouper at date of capture. Gag were collected at ingress (captured in bridgenet), during estuarine phase (captured in trawls, traps, and seines), and during egress (captured in channel net) from (A) 2007 and (B) 2008. Linear regression fits (C) to total length and date of collection for overlapping estuarine dates of collection in 2007 and 2008.

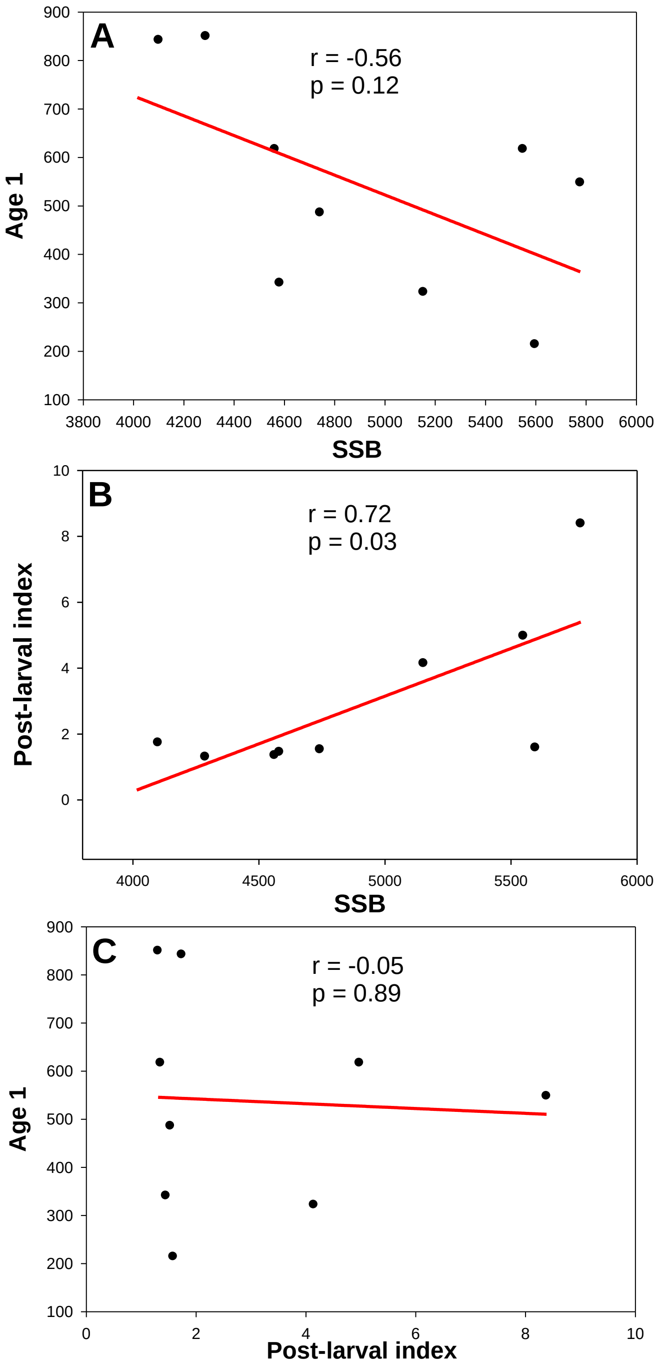

Figure 1.9. Pearson correlation analyses between (A) age-1 gag grouper (listed as age-0 in SEDAR10 (2007)) and spawning stock biomass (SSB) of gag grouper (lagged one year; SEDAR 10), (B) dglm adjusted post-larval index values (this study) and SSB of gag grouper, and (C) age-1 gag grouper and dglm adjusted post-larval index values (lagged one year; this study).

EARLY LIFE HISTORY OF POST-LARVAL AND JUVENILE GAG GROUPER IN NORTH CAROLINA

2.1 Introduction

Gag grouper are a commercially and recreationally important fish in the US south Atlantic. They range from Massachusetts to Brazil with the center of abundance thought to be in the Gulf of Mexico (Briggs, 1958; McErlean, 1963; Smith, 1971). In the US, two stocks are recognized and managed separately; one stock occurs in the South Atlantic Bight (SAB) ranging from North Carolina to southeast Florida and the other occurs in the Gulf of Mexico. Although not typically considered a highly migratory species, adults have been found to undertake spawning migrations of hundreds of kilometers, from South Carolina to Florida (Collins et al., 1996; Heinisch and Fable, 1999; McGovern et al., 2005). However, this type of migration is not common and most adult gag grouper typically have small home ranges (< 2km; Heinisch and Fable, 1999; McGovern et al., 2005).

Gag grouper spawn offshore (Collins et al., 1987) and eggs hatch within 2-3 days after fertilization (Roberts and Schlieder, 1983). Post-larvae ingress into estuaries and settle into seagrass beds (Ross and Moser, 1995; Koenig and Coleman, 1998) and into oyster reef habitat in South Carolina where seagrass is absent (Keener et al., 1988). Cold fronts or sudden drops in temperature in late summer/early fall are related to mass emigration of juvenile gag grouper out of estuaries and onto hard bottom ocean habitats although some emigration occurs throughout summer and into late fall (Ross and Moser, 1995; Koenig and Coleman, 1998; Chapter 1).

Gag grouper have experienced dramatic declines in abundance as a result of overfishing (McGovern et al., 1998; Harris and Collins, 2000). Because gag grouper form spawning aggregations, one management tool has been a spawning season closure. Current fishing closures in the US south Atlantic are centered around peak gag grouper spawning in March and April based on earlier gonad and fertilization date information (Collins et al., 1987; Keener et al., 1988). Amendment 16 of the South Atlantic Fisheries Management Council (SAFMC) proposed a closure beginning in January and continuing through April (SEDAR). Given the regional differences in fertilization and settlement dates observed in Florida (Fitzhugh et al., 2005), daily ages of post-larval and juvenile gag grouper collected in other US south Atlantic estuaries could help inform managers on spawning closures. Here, I estimated spawning dates of adult gag grouper from daily ages of post-larval and juvenile gag grouper collected in an estuary at the northern limit of their range; lunar periodicity in spawning activity was also examined. The effect of pelagic larval duration (PLD) on survival was examined by sampling juvenile gag grouper repeatedly throughout the summer. Because daily ages in otoliths become increasingly difficult to discern as juvenile fish get older (Ahrenholz, 1995), I estimated the age at which increments in gag grouper otoliths can be read with confidence. Lastly, growth rates were estimated from daily age and size information and examined for interannual variability.

2.2 Materials and Methods

2.2a *Study area*

This study was conducted in the area surrounding Cape Lookout, North Carolina. Post-larval gag grouper were sampled from the bridge (bridgenet sampling) leading to Pivers

Island at the NOAA fisheries laboratory located in Beaufort, NC (Figure 1; Chapter 1; Warlen et al., 1985; Hettler et al., 1998; Tzeng et al., 2003). Pivers Island is located in Back Sound, approximately 1.5 kilometers inside of Beaufort Inlet. Collections of juvenile gag grouper were made in Bogue, Back and Core sounds in areas with high salinity (> 30 ppt) (Figure 1). Ocean water is exchanged with these three sounds mainly through Beaufort Inlet (Churchill et al., 1999).

2.2b *Collection of samples*

A detailed description of gag grouper collections is provided in Chapter 1. Briefly, post-larvae were collected nightly in 2007 and 2008 using a 1m diameter ring net with 500 µm mesh. Post-larvae collected in NOAA's weekly ichthyoplankton sampling program (1x2m neuston net with 1mm mesh) from 1991 to 1998 and 2005 to 2006 were also included in this study; otoliths from these fish will be referred to as "archived otoliths". Samples were preserved in 95% ethanol and later sorted at the lab.

Juveniles were collected from June through September from seagrass beds using a 5m otter trawl with 12.7mm bar mesh in the body of the net and 3.2mm bar mesh in the bag. Juvenile gag grouper were also captured in a variety of other gears including beam trawl, minnow traps, and seine nets. In 2007, egressing juveniles were collected in channel nets in collaboration with commercial shrimp fishermen; no channel net catches were obtained in 2008.

2.2c *Age and growth preparation*

Ages (in days) were estimated from post-larval and juvenile gag grouper otoliths. Standard lengths (SL) and total lengths (TL) were recorded for post-larvae and juveniles. Total lengths were used for fish caught in 2007 and 2008 because TL measurements of juveniles were more precise than SL measurements. Standard lengths were used for analyses that included archived otoliths because TL measurements were not available. Sagittal and lapillar otoliths were removed and mounted on a slide. Lapilli were used to age all fish with the assumption that otolith increments are deposited daily; although a successful otolith validation study has not been conducted for gag grouper, past studies have assumed daily increments for post-larval and juvenile gag grouper (Keener et al., 1988; Rutten, 1998; Fitzhugh et al., 2005) given that this assumption has been validated in a variety of other reef fishes. Although sagittae are typically used in most aging studies, lapilli require less preparation and have been used frequently to age post-larval and juvenile gag grouper (Keener et al., 1988; Rutten, 1998, Fitzhugh et al., 2005). Lapilli from post-larvae were mounted on a slide in Depex TM mounting medium and read whole. Lapilli from juveniles were mounted on a slide in Depex TM and read after a frontal polish because of the greater thickness of otoliths in juveniles. Most juvenile otoliths could be read after polishing only one side, others had to be polished on both sides. Daily growth increments were counted in two separate steps for juveniles: counts were made inside the transition mark (IT) and outside the transition mark (OT). The transition mark is a change in the optical density and coloration of the otolith that occurs in many reef species and represents the period of transition from pelagic post-larvae to benthic juveniles (Figure 2; Keener et al., 1988). The

IT count was assumed to represent PLD though Keener et al. (1988) observed transition marks on ingressing post-larvae. A minimum of two counts per zone were done for each otolith. If the first two readings were within ten percent of each other, an average of the two counts were used to determine age. If the first two counts were not within ten percent, a third reading was done. If the third count was within ten percent of either of the two previous counts, the two most similar counts were then averaged. If there was not at least ten percent agreement after three readings, the otolith was discarded.

Otoliths were read using an Olympus BX41 compound microscope at 200X for OT counts and 1000X under oil immersion for IT counts; a camera was mounted on the microscope and otoliths were read using image analysis software (Olympus Microsuite 8 Basic Edition) on a 30" LCD monitor. Adobe PhotoShop Elements was used to enhance still images for easier reading.

Average daily growth increments counts from IT and OT were added together to obtain the uncorrected age. To obtain fertilization dates, a correction factor of six days was added to the uncorrected age following Keener et al. (1988). The six days account for an assumed two to three days between fertilization and first increment formation (Roberts and Schlieder, 1983) and three days for increments that were observed in sagittae but not observed in lapilli. The latter assumption was based on unpublished data cited in Keener et al. (1988). To confirm this, I compared post-larval ages using sagittae and lapilli that were taken from the same fish and found an average of 3.25 days difference between the two structures (n = 48; sagittae: mean = 44.93 ± 5.79 SD; lapilli: mean = 41.68 ± 4.96 SD).

Otoliths from all post-larvae caught in 2007 and 2008 were aged; archived otoliths from post-larvae were also aged.. Juvenile gag grouper caught in 2007 and 2008 were sub-sampled; a minimum of two fish per 1cm (TL) increment were aged each year giving equal numbers of juvenile otoliths read in each year.

2.2d *Data analyses*

One-way ANOVA was used to examine for an effect of collection month on fertilization dates and PLD in 2007 and 2008. Tukey's HSD was used as a post-hoc test. Relationships between PLD and fertilization date, ingress date, and SL were investigated using Pearson product-moment correlation.

Lunar periodicity in successful spawning dates was analyzed using periodic regression terms (deBruyn and Meeuwig, 2001) for both post-larval and juvenile back-calculated fertilization dates. Fertilization dates from 2007 and 2008 data were analyzed alone because gag grouper post-larvae were collected nightly and juveniles were caught throughout the summer. Fertilization dates were also analyzed with all data (including archived otoliths). Data included in this analysis was limited to post-larvae and juveniles collected through July because of suspected underaging of fish beginning in August. Lunar days were defined as days from full moon and were based on a 29 day lunar cycle (full moon = 0, new moon = 14.5). The cosine term describes peaks at either new or full moons, the cosine 2θ term describes peaks at both new and full moons. The sine term describes peaks at either the first and third quarter of a lunar cycle, while sine 2θ describes peaks at both the first and third quarters. Models that include both a θ and 2θ term can have two peaks per lunar cycle that are unequal in amplitude (deBruyn and Meeuwig, 2001). A generalized linear modeling approach was used to model number of gag grouper as a function of the above independent variables assuming a poisson distribution and a log link; analyses were done using proc genmod (SAS) and AICc (Burnham and Anderson, 2002) for model selection. AICc values were used because of small sample size.

2.2e *Growth rates*

Growth rates of juvenile gag grouper were estimated during the summer months (June to September) using estimated age (days) from otoliths and total length (TL). Post-larval gag grouper were not included in the growth analyses because size did not significantly vary with age at ingress ($r^2 < 0.01$, $p=0.91$). Although gag grouper are known to grow rapidly in warm waters until egress (Chapter 1), fall caught juveniles were not included in this analysis because gag grouper caught later than September could not be reliably aged (see below). Linear regression was used to estimate average growth for 2007 and 2008. Growth rates between years were compared using a homogeneity of slopes test.

2.3 Results

A total of 197 otoliths were aged successfully. For 2007, estimated ages were possible from 55 juveniles and 19 post-larvae. Daily ages from 55 juveniles and 14 post-larvae were estimated from 2008 collections. Fifty-four post-larval otoliths provided daily age estimates from the archived samples.

There was an effect of collection date on fertilization date in both 2007 and 2008. However, back-calculated fertilization dates from fish collected in May and July were not significantly different ($p > 0.05$, Figure 2.3A); fertilization dates ranged from March through April. Fertilization dates of fish caught after July were significantly different than those caught in the bridgenet (May). Estimated fertilization dates from gag grouper caught in October and November of 2007 were considered to be invalid based on the absence of these late fertilization dates in earlier samples and the nonsensical estimates of ingress date. These fish were assumed to be underaged and excluded from all further analyses except analyses of PLD. In 2008, back-calculated fertilization dates from May, June and July collected gag were not significantly different ($p > 0.05$, Figure 3B); all fertilization dates were in March and April. Mean fertilization dates estimated from gag grouper collected after July were significantly later in the year compared to earlier collections. However, median fertilization dates for August and September were very similar to those of May through July in both 2007 and 2008. Thus, fish from April through September were used for analyses of growth rate (juveniles from June-Sept) and when pooling fertilization dates. Fertilization dates from post-larvae collected in April 2008 were from a spawning event in February 2008 (Figure 3B); February fertilization dates were not represented in later collections.

Back-calculated fertilization dates of post-larval gag grouper (data from 2007 and 2008 plus archived otoliths) ranged from February through April with peaks in March (Figure 2.4A). Archived otoliths were collected only through April in most years (see Table 1.1 in Chapter 1); fertilization dates from those collections may be biased towards earlier fertilization dates when compared to post-larvae collected in both April and May of 2007 and 2008 (Figure 2.4A). Post-larval data were used to compare fertilization date and ingress date; ingress occurred on average ~45 days after fertilization (Figure 2.5). Back-calculated fertilization dates for juvenile gag grouper caught from June through September ranged from mid-March to late April (Figure 2.4B) and the combined fertilization data from both larvae and juveniles shows a spawning period from February to late April (Figure 2.4C).

Collection month did not have a significant effect on PLD in gag grouper in 2007 ($p = 0.81$; Figure 2.6A) or 2008 ($p = 0.23$; Figure 2.6B). Mean PLD in 2007 was 43.71d while mean PLD in 2008 was 44.97d; given similarities in each year, data were combined into one plot (Figure 2.6C).

Pelagic larval durations of post larvae were variable at ingress but size was not (2007: age range 32-52 d, TL range 15-21 mm; 2008: age range 34-61 d, TL range 17-22 mm). Post-larval gag grouper that were from later fertilization dates had shorter PLDs (Figure 2.7A; $r = 0.13$, $p < 0.001$). However, there was no relationship between PLD and post-larval ingress date (Figure 2.7B, $p=0.68$) or post-larval size (Figure 2.7C; $p = 0.31$).

Periodicity in successful spawning activity was evident (Figure 2.8A & B). In 2007 and 2008, most back-calculated fertilization dates were centered around full moons with minimal spawning occurring around new moons; there was equal support for a model that described fertilization at dates near full moons (model 1a; Table 2.1a) and at dates near full moons with a smaller peak after the new moon (model 1b; Table 2.1A; Figure 2.8A). However, when post-larval ages from archived otoliths were added, there was equal support for models 1b-4b that varied greatly in their predicted peaks in fertilization (Table 2.1B; Figure 2.8B). Although AICc model selection was not able to distinguish a best model from this combined data, there is periodicity in fertilization dates. The model with the lowest ΔAICc ($\cos 2\Theta$) describes equal peaks in successful spawning activity around full and new moons. The other three models with equal support describe a nearly random distribution of spawning activity throughout the lunar cycle.

Juvenile gag grouper grew relatively fast (2007 = 1.8 mm/d; 2008 = 1.6 mm/d) during the summer (Figure 2.9). Growth rates (slopes) did not differ between years (homogeneity of slopes test; $p = 0.70$).

2.4 Discussion

Counting otolith increments in older, larger juvenile fish is difficult because the outer rings often coalesce (Ahrenholz, 1995). At that point and beyond, the number of daily growth increments is no longer a reliable indicator of age. The size and age at which this occurs is species-specific and dependent upon the microstructure of the otolith. Ahrenholz et al. (1995) found that Atlantic menhaden could be reliably aged up to 200 days using sagittal otoliths. Based on Ahrenholz's (1995) finding, Fitzhugh et al. (2005) aged juvenile gag grouper caught through July in the Gulf of Mexico; those juveniles were presumed to be less than 200 days old based on assumed spawning period.

Back-calculated fertilization dates shifted to later dates beginning with August collections; this provides evidence of underaging as the later fertilization dates were not represented in earlier samples. However, some of the shift in fertilization dates at later collections could be due to ontogenetic habitat shifts (see Ross and Moser (1995)) of older/larger juveniles out of the grass beds. Rutten (1998) back-calculated fertilization and ingress dates using lapilli from juvenile gag grouper caught from 26 August to 29 September 1997 in New River, NC. His estimated fertilization dates peaked in April to early May with ingress (estimated by adding the PLD to fertilization date) centered in early June. Rutten's (1998) estimated fertilization and ingress dates are biased ~ 20 to 30 d later when compared to estimated fertilization dates from younger, smaller gag grouper and observed for ingress in NC and SC (Keener et al., 1988; this study). Given the evidence for underaging in this study and inferred from Rutten's (1998) study, I conclude that daily ages from juvenile gag grouper are unbiased through July (up to ~145 d old fish). However, since the majority of fertilization date estimates from gag collected in August and September in this study were realistic, I retained those ages in the pooled fertilization date graph and in the growth rate analyses.

Fitzhugh et al. (2005) collected juvenile gag grouper from multiple locations along a latitudinal gradient on the west coast of Florida; they found regional differences in fertilization and settlement dates of gag grouper within this limited geographic range. Given this latitudinal effect, I investigated whether spawning times of gag grouper that provide post-larvae to North Carolina (northern limit of their range) differed from a previous study conducted in South Carolina (Keener et al., 1998). I found successful spawning activity to occur from February through April with peak activity occurring from early March to early April. This is consistent with Keener et al.'s (1988) findings and from histological analyses of gonads (Collins et al., 1987). Peak spawning of gag grouper in the Gulf of Mexico occurs earlier (February through March; McErlean, 1963; Collins et al., 1987; Koenig et al., 1996; Fitzhugh et al., 2005) possibly due to differences in water temperature.

Gag grouper form annual site-specific spawning aggregations providing fishers with a potential for high catchability (McGovern et al., 1998; Gilmore and Jones, 1992). Because of this, spawning closures are thought to be effective at maintaining spawning stock biomass; these temporal closures are currently being lengthened from March through April to January through April. Only indirect methods have been used to assess spawning activity of gag grouper because spawning events have never been observed directly (Koenig et al., 1996). Fitzhugh et al. (2005) found evidence of successful spawning activity in January and February in Florida. My information on spawning periods provides further justification for a February spawning season closure in the US south Atlantic.

Gag grouper from all collection months were used for analysis of PLD because aging error does not affect counts within the transition zone (Ahrenholz, 1995). Pelagic larval duration of gag grouper recruiting to Florida and the Carolinas has consistently been found to be ~45 days (Keener et al., 1988; Rutten, 1998; Fitzhugh et al., 2005; this study). Gag grouper have a relatively long PLD (~45 days) when compared to other *Mycteroperca spp.* on the west coast (~24 days; B. Victor, pers. comm.). This could be related to distance to offshore spawning grounds; off NC, spawning grounds are at least 50km offshore while congenerics in the eastern Pacific spawn much closer to shore. PLDs in other reef fish species are related to oceanographic conditions (e.g. water temperature) and growth rate (Searcy and Sponaugle, 2000, Sponaugle and Grorud-Colvert 2006). Gray snapper (a reef species that shares a similar life history with gag grouper) caught at the Pivers Island bridgenet site in warmer summer months were found to have a mean PLD of only 27 days, yet the nearest known spawning grounds are 100s of kms to the south (Tzeng et al., 2003). A trend towards shorter PLDs in post-larval gag grouper spawned later in the season is likely due to water temperature (effects on larval development) but distance cannot be ruled out (effect of water temperature on spawning).

Gag grouper had higher variability in PLDs than in size at ingress; thus, larval growth rates were highly variable. Similarly, Cowen (1991) found that age at settlement for California sheephead ranged from 37 to 78 days, yet size ranged only from 12.7 to 16mm. Through examination of otolith increment widths, he determined that fish with long PLDs had an abrupt slowing of growth after 35 to 37 days in the plankton with continued slow growth until settlement. Conversely, fish that settled early (e.g. 35 to 40 days) had no evidence of slow growth. Wellington and Victor (1992) also found that faster growing reef fishes spent less time in the plankton than slower growing fish. Cowen (1991) determined that post-settlement growth was not affected by the presence of a slow growth period, suggesting that long PLDs were not detrimental to the survival of the fish. I tested the hypothesis that gag grouper with longer PLDs have lower post-settlement survival (i.e., 'stage duration hypothesis'; Houde 1987). I did not have estimates of survival to test this hypothesis directly; instead, I examined PLDs of juvenile fish collected monthly after settlement to test whether fish with longer PLDs were selectively removed over time. I found no evidence for this hypothesis as mean PLD did not change with month of collection. PLDs observed in post-larval gag grouper were also observed in late-stage juveniles collected up to 180 days after settlement.

Successful gag grouper spawning in 2007 and 2008 peaked around full moons with a lesser peak around new moons. Adding the archived otoliths produced a peak in fertilization dates around new moons nearly equal to that around full moons; this was mostly due to a single large pulse of post-larval gag grouper that was caught on a single night (n = 26, 21

April 1994) near a full moon. Perhaps if otoliths from 1999 through 2004 were available for this study, a larger proportion of successful spawning activity would be evident around new moons (as in Figure 2.8A). Keener et al. (1988) also noted periodicity in successful spawning activity around new and full moons based on ages of post-larval gag grouper ingressing into a South Carolina estuary. For many reef fishes, spawning activity is related to times of full moons with secondary peaks at new moons (Findlay and Allen, 2002; Walsh, 1987).

Gag grouper residing in estuaries have been described as a single cohort in both the eastern and western Gulf of Mexico (Koenig and Coleman, 1998; Renan et al., 2006) as well as in North Carolina (Ross and Moser, 1995). The consistency of fertilization dates over several months and the low variability in size by capture date (Chapter 1) support these prior studies; gag grouper recruit to North Carolina estuaries as a single cohort that are spawned from February to April. Interestingly, the February fertilization dates determined from the pulse of gag grouper post-larvae caught in April of 2008 were not observed in subsequent collections. Two plausible explanations for the lack of February fertilization dates in juvenile collections are: (1) February spawned gag grouper do not survive once settling in the estuary, or (2) successful spawning in February is rare and there is a low probability of catching them once settled. Further work is needed to determine if February spawned gag survive into the juvenile stage.

Growth rate estimates using size and otolith age were rapid at ~1.7 mm/d and were very similar to growth rates estimated using size and capture date (see Chapter 1). However, capture date explained slightly more variability (r^2= 0.70 and 0.88) in juvenile gag grouper size compared to daily age (r^2=0.69 and 0.81). This is most likely a result of juvenile gag grouper starting at a similar size at a similar settlement date within a year but with a larger range in age. Aging error likely explains some of the increased variability in aged-based growth rates as well. Juvenile gag grouper collected from oyster reef habitats in South Carolina were found to grow slightly slower at ~1.33mm/day (Mullaney and Gale, 1996) and there was a wide range in growth rates from different Florida estuaries (1.07 to 1.85mm/d; Strelcheck et al., 2003). Juvenile gag grouper growth rates are similar to other juvenile estuarine fish that have diets dominated by fish prey (see Juanes and Conover 1995 for review); Ross and Moser (1995) found diets of juvenile estuarine gag grouper to be predominantly fish.

I found that post-larval and juvenile gag grouper collected in North Carolina shared early life history characteristics with those described for South Carolina and Florida. Spawning activity that results in successful recruitment to both North Carolina and South Carolina takes place primarily in March and April and to a lesser degree in February around new and full moons. Pelagic larval duration in all three states averages ~ 43 days; in NC, there was no evidence for an influence of PLD on survival to the late juvenile stage. I was unable to confidently age juvenile gag grouper in fall months due to underaging which most likely resulted from coalescing of outer rings. Juvenile gag grouper grow rapidly while residing in North Carolina seagrass beds and this habitat should be protected for its nursery function. The results of this study support the South Atlantic Fishery Management Council’s Amendment 16 (SEDAR 10, 2007) to add January and February to the current March and April spawning season closure.

Table 2.1. (A) Models describing fertilization dates throughout a lunar cycle ranked by ΔAICc for 2007 and 2008 only. (B) Models describing fertilization dates throughout a lunar cycle ranked by ΔAICc with data from all years included (1991-1998, 2005-2008). Models with ΔAICc values < 2 have equal support.

A

ID	Model	AICc	ΔAICc
1a	gag = cos(Θ) + sin(2Θ)	-8.62	0.00
2a	gag = cos(Θ)	-6.92	1.70
3a	gag = sin(2Θ)	-4.46	4.16
4a	gag = sin(Θ)	-0.64	7.98
5a	gag = cos(2Θ)	-0.26	8.36
6a	gag = sin(Θ) + cos(2Θ)	1.88	10.50

B

ID	Model	AICc	ΔAICc
1b	gag = cos(2Θ)	-173.98	0.00
2b	gag = sin(2Θ)	-172.88	1.10
3b	gag = cos(Θ)	-172.80	1.18
4b	gag = sin(Θ)	-172.70	1.28
5b	gag = sin(Θ) + cos(2Θ)	-171.28	2.70
6b	gag = cos(Θ) + sin(2Θ)	-170.30	3.68

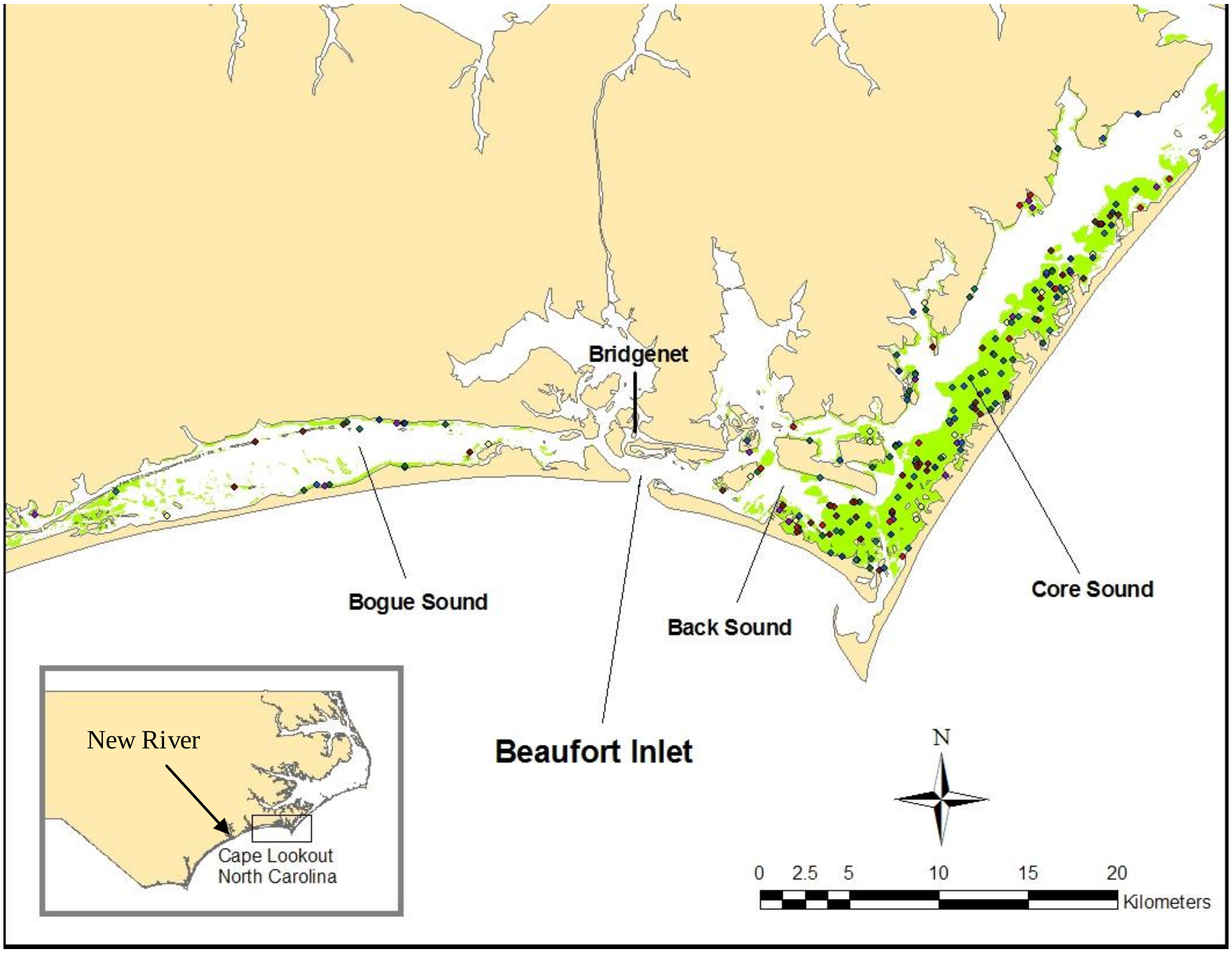

Figure 2.1. Map of the study site around Cape Lookout, NC. Bridgenet is located ~1.5km inside of Beaufort Inlet. Shaded areas inside of Bogue, Back, and Core sounds represent seagrass beds mapped by NOAA. Points inside of seagrass beds represent randomly generated trawl locations.

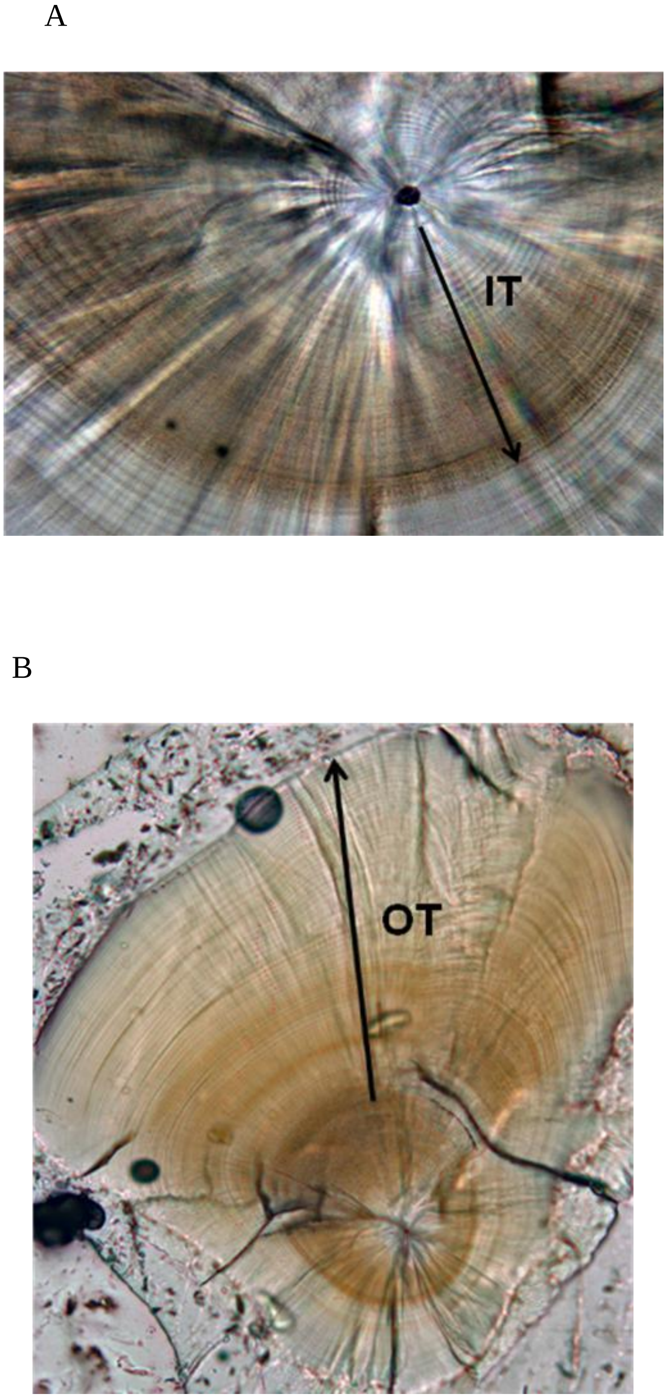

Figure 2.2. Microstructure of a lapillus from juvenile gag grouper. (A) IT zone (representing PLD) shown at 100X under oil immersion. (B) General view of a lapillus under 20X magnification.

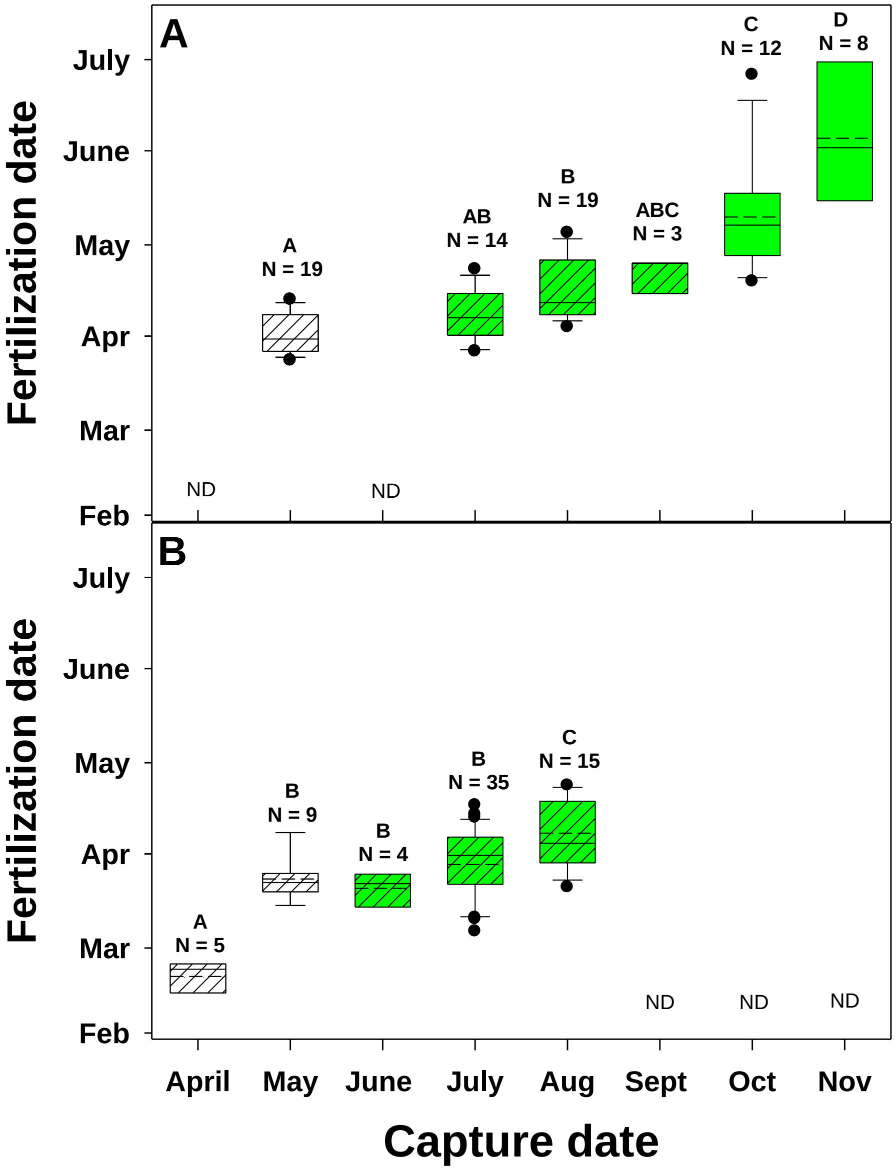

Figure 2.3. Fertilization date distributions of gag grouper by month of capture in (A) 2007 and (B) 2008. Light boxes represent fish caught as post-larvae in bridgenet. Dark boxes represent fish caught as juveniles. Cross-hatched boxes represent months for which fish were confidently aged. Dashed line = mean; solid line = median; bottom boundary of box = 25th percentile; top boundary of box = 75th percentile; whiskers below and above box (error bars) = 10th and 90th percentile, respectively; closed circles = outliers; ND = no data.

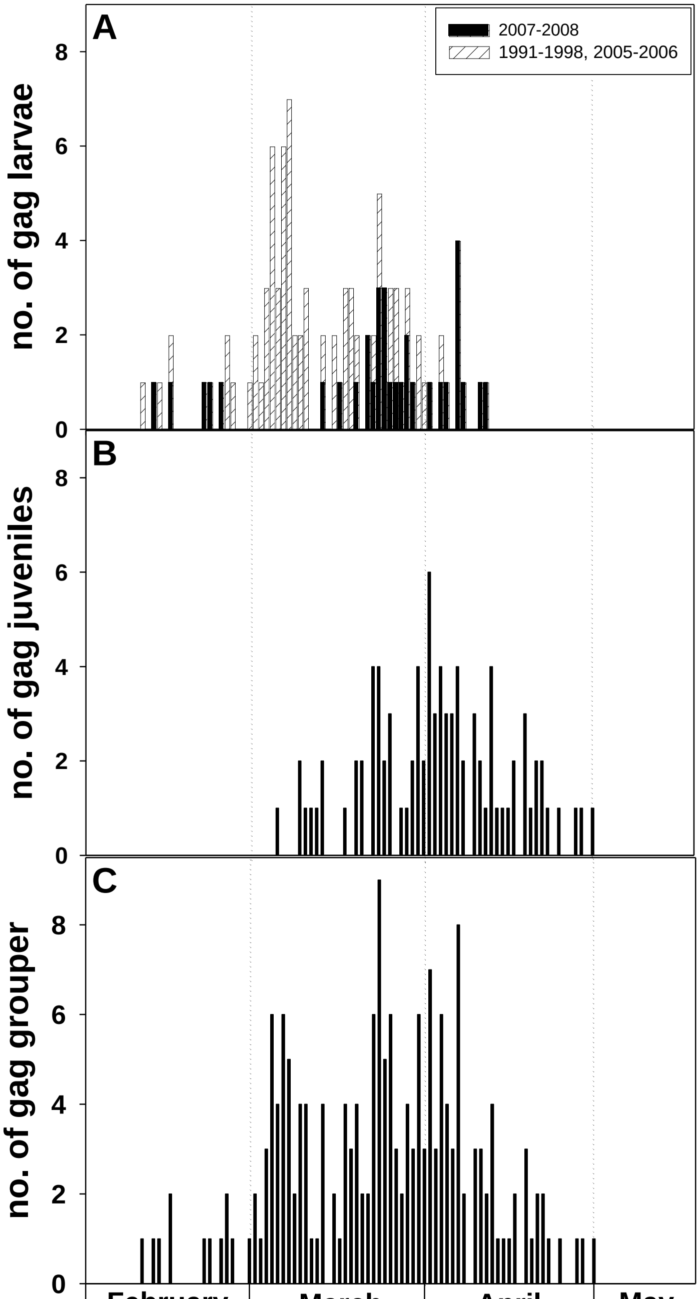

Figure 2.4. Fertilization dates of gag grouper: (A) post-larvae where dark bars represent collections during nightly sampling (2007-2008) and hatched bars represent collections during weekly sampling (1991-1998, 2005-2006); (B) juveniles sampled in this study; and (C) post-larvae and juveniles from all years combined.

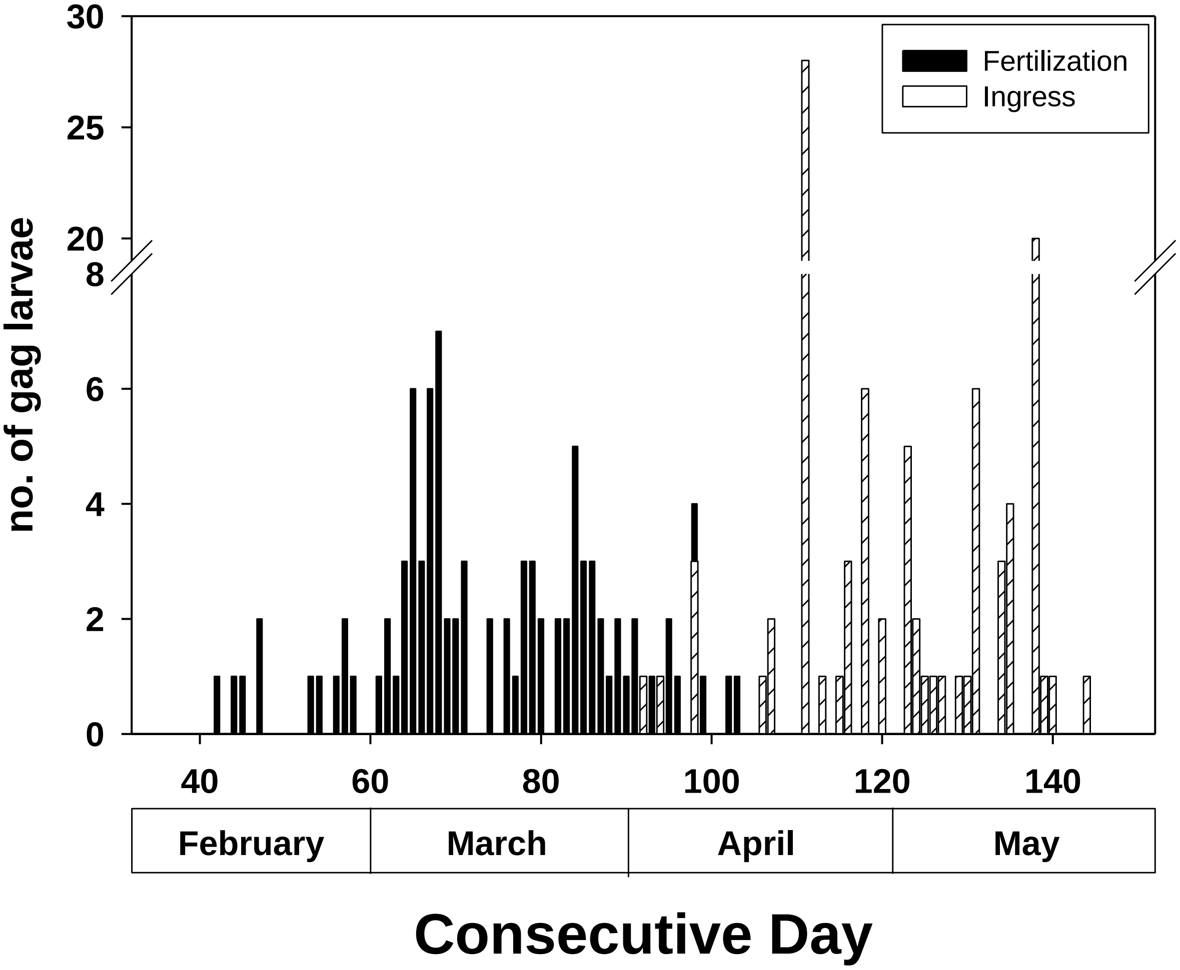

Figure 2.5. Fertilization (dark bars) and ingress (light bars) date distributions for post-larval gag grouper collected in the bridgenet at Pivers Island, NC from 1991-2008. Consecutive days start with 1 January = 1.

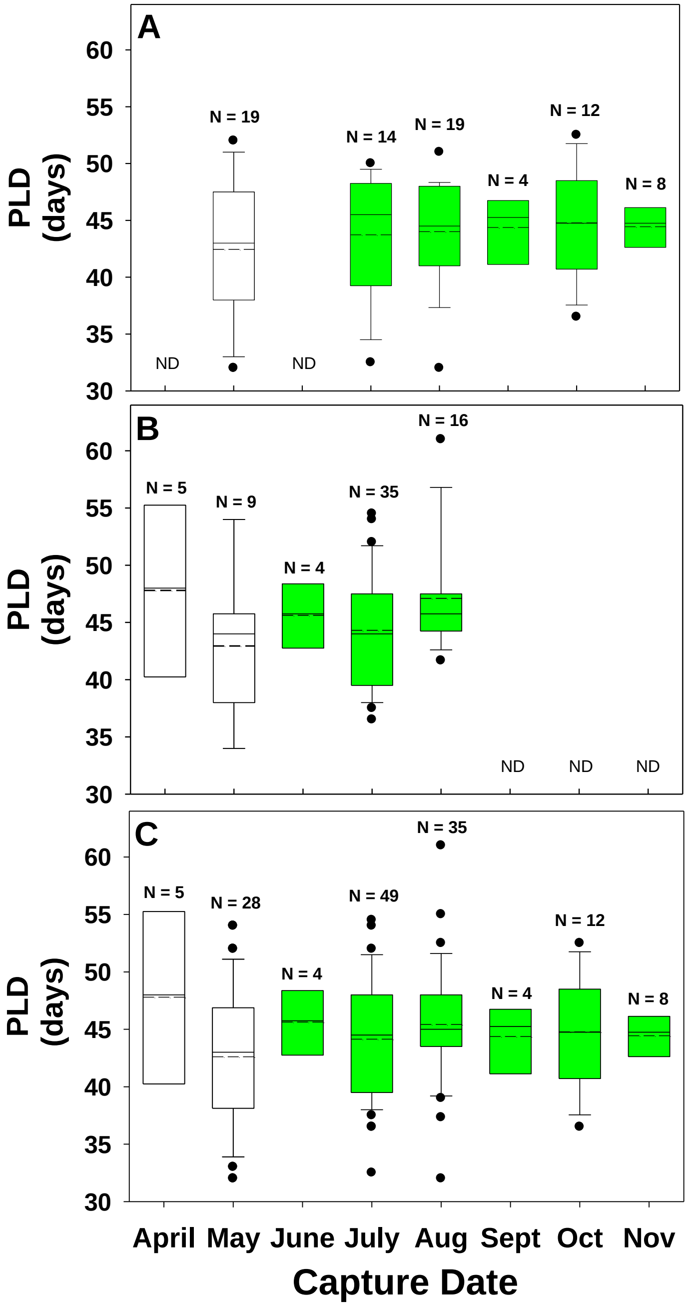

Figure 2.6. Pelagic larval duration distributions of gag grouper by month of capture in (A) 2007, (B) 2008, and (C) 2007 and 2008 combined. White boxes represent fish caught as post-larvae in bridgenet. Dark boxes represent fish caught as juveniles. Dashed line = mean; solid line = median; bottom boundary of box = 25th percentile; top boundary of box = 75th percentile; whiskers below and above box (error bars) = 10th and 90th percentile, respectively; closed circles = outliers; ND = no data.

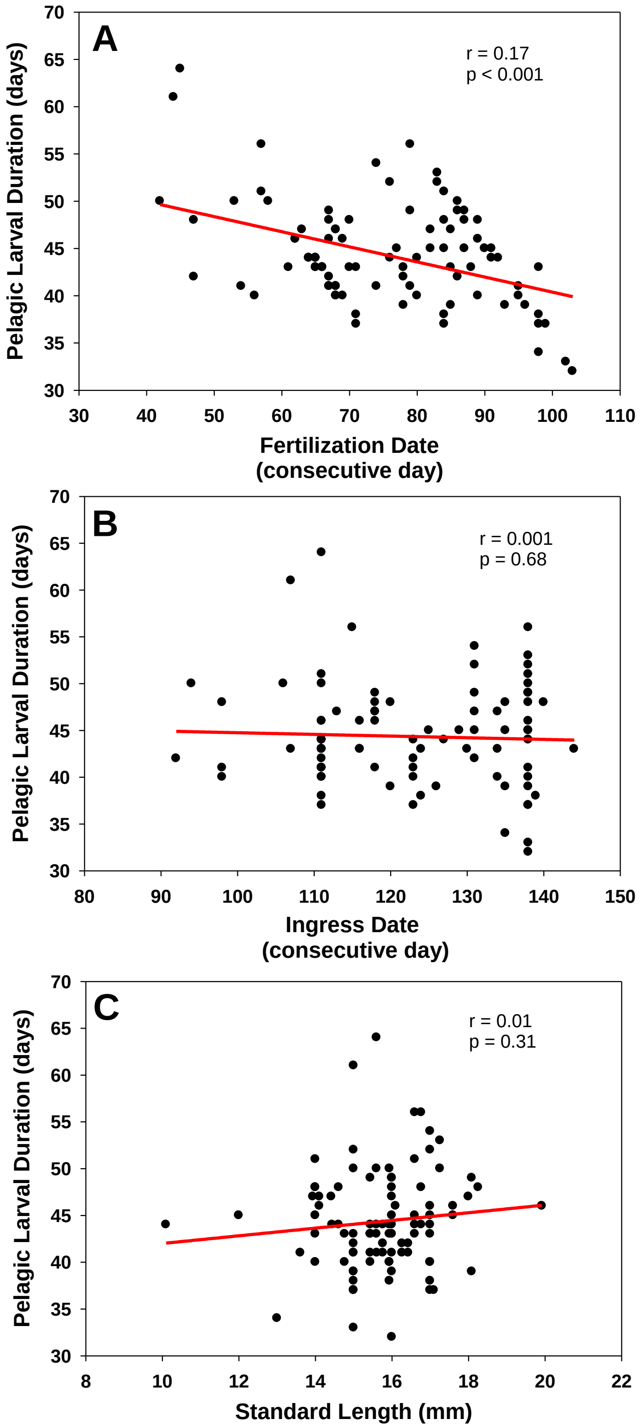

Figure 2.7. Pelagic larval durations of post-larval gag grouper from 1991-2008 as a function of (A) fertilization date, (B) ingress date, and (C) standard length. Relationship is significant only for fertilization date. Consecutive days start with 1 January = 1.

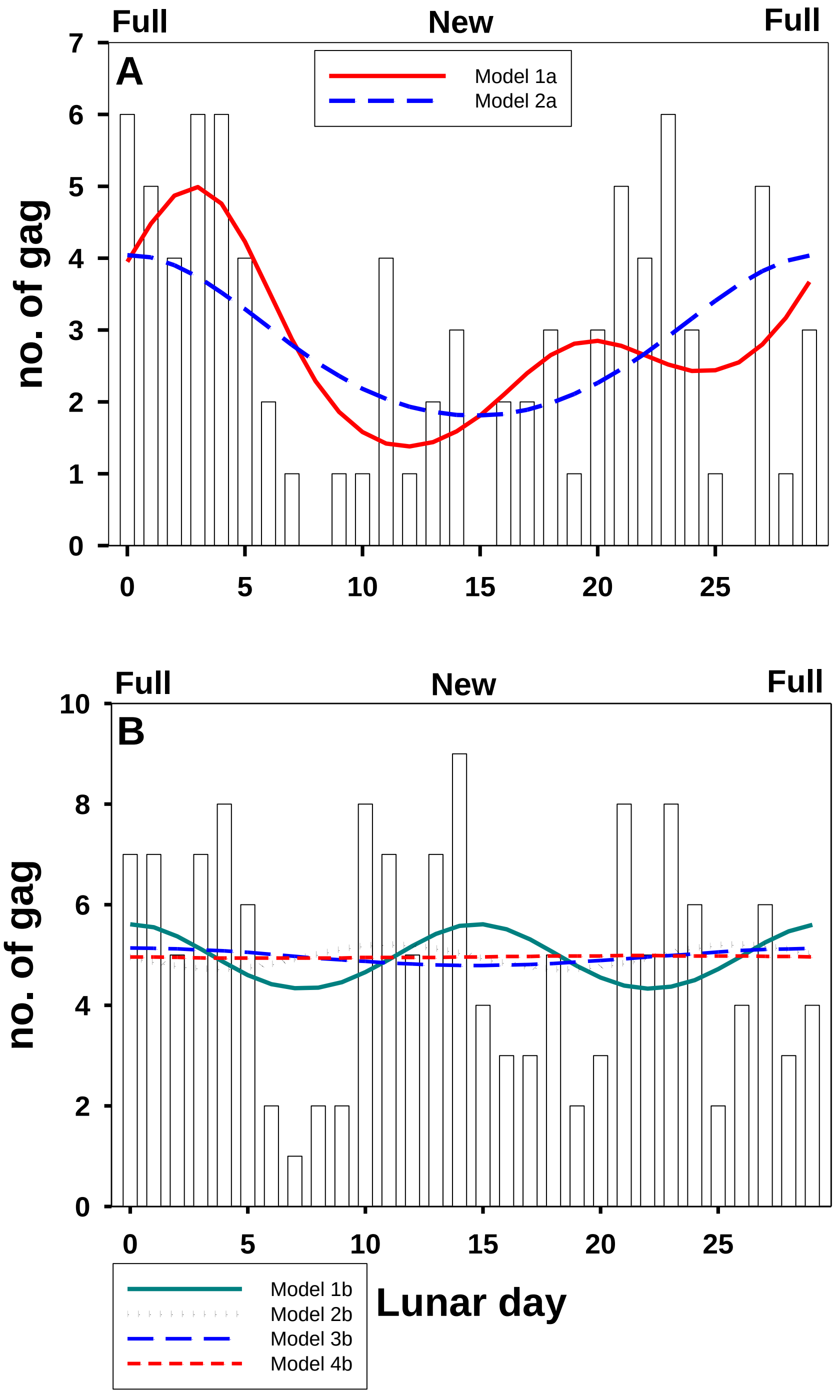

Figure 2.8. Fertilization dates as a function of lunar day for post-larval and juvenile gag grouper caught at ingress and through July in (A) 2007 and 2008 and (B) the 2007 and 2008 data with fertilization dates for post-larvae collected from 1991-1998 and 2005-2006. The predictions from the top models with equal support under ΔAICc (see Table 1) are plotted with the observed data.

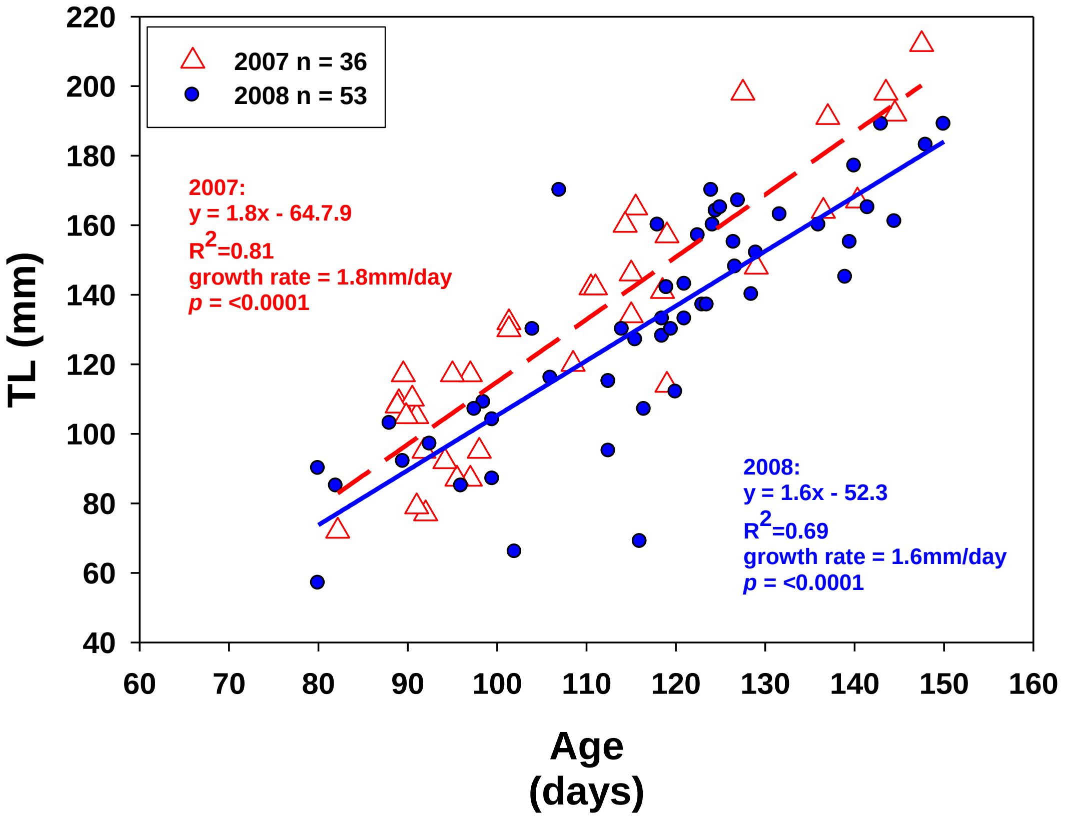

Figure 2.9. Total lengths of juvenile gag grouper at age collected from June through September in 2007 and 2008. Lines represent linear regression fits (details on graph) for each year.

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