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FERTILIZATION DATES, PELAGIC LARVAL DURATIONS, AND GROWTH IN GAG (*MYCTEROPERCA MICROLEPIS*) FROM NORTH CAROLINA, USA

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ABSTRACT

We estimated pelagic larval duration (PLD) and age from the otolith microstructure of post-larval and juvenile gag, *Mycteroperca microlepis* (Goode and Bean, 1879). These estimates were used to: (1) estimate spawning periods; (2) evaluate lunar periodicity in spawning; (3) assess relationships between PLD and fertilization date, ingress date, capture date, and size at capture; and (4) compare juvenile growth rates in two consecutive years and with rates determined in previous studies. Postlarval and juvenile gag were collected from late spring to early fall of 2007 and 2008 using a variety of gear types; otoliths from postlarvae collected in previous years were also examined. Estimated fertilization dates ranged from February to April with concentrations aligned with the first and third quarters of the lunar cycle. The mean PLD was approximately 45 d for fish collected as postlarvae or juveniles despite a 6 mo range of collection dates (April–September). The distributions of PLDs were similar among sampling months suggesting no effect of PLD on subsequent survival. Although there was no relationship between PLD and date of ingress, PLD was shorter for fish with later fertilization dates. Juvenile growth rates derived from length and estimated ages were approximately 1.4 mm d^{-1} during summer months and did not differ between years. Our findings support the timing (January–April) of fishing closures on aggregations of spawning gag in the southeast US and suggest that post-settlement survival is not linked to PLD.

The otolith microstructure of larval and juvenile fishes has been used to address a variety of basic and applied questions related to the biology and ecology of fishes (Cowen and Sponaugle 1997, Thorrold and Hare 2002, Sponaugle 2010). Back-calculated hatch dates have improved our ability to determine spatiotemporal patterns in adult spawning and cohort structure, which has proved useful in management of exploited fishes (Brophy et al. 2006). Additionally, a variety of questions related to recruitment in reef fishes have been addressed for species that exhibit settlement marks. For example, Searcy and Sponaugle (2001) found evidence that mortality of recently settled reef fish was negatively related to larval growth and positively related to pelagic larval duration (PLD).

Gag [family Serranidae, subfamily Epinephelinae, *Mycteroperca microlepis* (Goode and Bean, 1879)] is a commercially and recreationally important reef fish found in association with hard-bottom shelf habitats in the southeast US large marine ecosystem (SEUS), which is a temperate to subtropical ecosystem extending from North Carolina to southeast Florida. In the US, two stocks of gag are recognized and managed separately; one stock occurs in the SEUS and the other occurs in the Gulf of Mexico ranging from southwest Florida to the US/Mexico border. In the SEUS, gag spawn from February to April (Collins et al. 1987) and fertilized eggs hatch within

2–3 d (Roberts and Schlieder 1983). Postlarval gag ingress into estuaries and settle in seagrass beds (Ross and Moser 1995, Koenig and Coleman 1998) and oyster reef habitat (Keener et al. 1988, Mullaney and Gale 1996). Keener et al. (1988) used the term *postlarva* to describe a transition-stage pre-settlement gag larva that lacked juvenile pigmentation. There is good evidence that the juvenile stage is dependent on estuarine habitats (Keener et al. 1988, Ross and Moser 1995), but whether this dependency is facultative or obligatory is unclear (see Able 2005). Juvenile gag grow in length >1.0 mm d^{-1} in SEUS estuaries during summer (Keener et al. 1988, Ross and Moser 1995, Mullaney and Gale 1996) and emigrate to shelf hard-bottom habitats in late summer to early fall (Ross and Moser 1995, Koenig and Coleman 1998, Adamski et al. 2011).

Gag adults have declined in abundance as a result of overfishing (McGovern et al. 1998, Harris and Collins 2000, SAFMC 2009). Because gag form spawning aggregations, which were targeted by fishers, spawning season closures were implemented in an attempt to reduce fishing mortality. Recent fishing closures in the SEUS were timed to align with peaks in gag spawning frequency, which have been estimated to occur in March and April based on earlier gonad examinations and estimated fertilization dates from otoliths of ingressing gag in South Carolina estuaries in the 1980s (Collins et al. 1987, Keener et al. 1988). The fishing closure was recently expanded to January–April in an attempt to further reduce fishing mortality on adult gag (Snapper Grouper Amendment 16, SAFMC 2009). Fitzhugh et al. (2005) reported differences in gag fertilization and settlement dates by estuarine region within Florida; estimates of the timing of these events for gag in SEUS estuaries outside of South Carolina are needed. Further, such additional data may allow determination of changes in spawning season over recent decades.

The transition between larval and juvenile stages of reef fishes, and the concurrent transition from pelagic to benthic habitats, is often a time of high mortality (Victor 1986a, Almany and Webster 2006). Searcy and Sponaugle (2001) showed that faster growing (and/or shorter PLDs) bluehead wrasse, *Thalassoma bifasciatum* (Bloch, 1791), larvae had higher survival through the juvenile stage; they hypothesized the bulk of selective mortality on settlers happened immediately after settlement. Other studies on reef fishes have found similar results (Vigliola and Meekan 2002, Hawn et al. 2005), but most of these studies were conducted in coral reef habitats where water temperature and fish prey productivity are unlikely to vary as much as in more temperate environments (Sponaugle and Grorud-Colvert 2006). For reef fishes with planktonic offspring whose presence coincides with periods of environmental variability (e.g., vernal warming or autumnal cooling), we might expect larval growth rates and PLDs to be more variable than observed for tropical species (Sponaugle and Grorud-Colvert 2006). This increased variability in growth or life-period duration might lead to a higher likelihood of selective mortality.

The objective of the present study was to estimate the PLD and age of postlarval and juvenile gag captured in North Carolina and use that information to address questions related to the biology and management of gag. The questions are: (1) Can gag be aged throughout the juvenile stage using lapillar otoliths? (2) Do gag from the northern part of the SEUS in the 2000s have different fertilization dates than gag caught farther south in the early 1980s? (3) Are gag fertilization dates related to the lunar cycle? (4) Is PLD related to survival of transitional and young juvenile gag? (5) Is PLD related to fertilization date, ingress date, or fish size? (6) Does inter-annual variability in growth rate occur in juvenile gag?

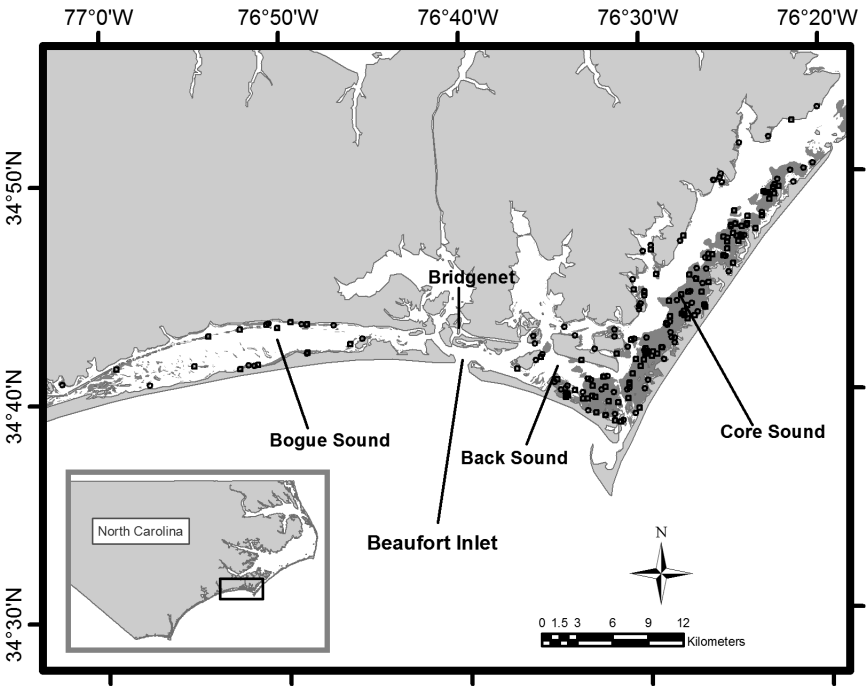

Figure 1. Map of the study area around Cape Lookout, North Carolina, USA. The bridgenet sampling platform is located approximately 1.5 km inside of Beaufort Inlet. Seagrass beds are represented as shaded areas inside of Bogue, Back, and Core Sounds. Points inside seagrass beds represent randomly generated stations where otter trawls were conducted in 2007 (circles) and 2008 (squares).

Methods

Study Area.—Samples for our study were collected near Cape Lookout, North Carolina (Fig. 1). Postlarval gag were collected by net from a bridge (bridgenet sampling) leading to Pivers Island at the NOAA (National Oceanic and Atmospheric Administration) Laboratory located in Beaufort, North Carolina. Pivers Island is located in Back Sound, approximately 1.5 km inside of Beaufort Inlet. Juvenile gag were collected by various means in seagrass beds or other structured habitats within Bogue, Back, and Core sounds (Fig. 1). Ocean water is exchanged with these three sounds mainly through Beaufort Inlet (Churchill et al. 1999).

Collection of Samples.—Postlarvae were collected by nightly bridgenet sampling during springs of 2007 (May–June) and 2008 (April–May) using a 1-m diameter ring net with 500-μm mesh. Postlarvae collected in NOAA's ichthyoplankton bridgenet sampling program from 1991 to 1998 and 2005 to 2006 were also included in the present study; otoliths from these fish are referred to as "archived otoliths." The NOAA bridgenet program samples weekly beginning in fall (approximately November) and ending in spring (April or May) using a 1 × 2-m neuston net with 1-mm mesh (see Warlen and Chester 1985, Hettler and Hare 1998, Tzeng et al. 2003 for further information on NOAA's bridgenet sampling program). Postlarvae from both sampling efforts were preserved in 95% ethanol and later sorted at the laboratory.

Juvenile gag were collected from June to September of 2007 and 2008 at randomly selected stations in seagrass beds using a 5-m otter trawl with 12.7-mm bar mesh in the body of the net and 3.2-mm bar mesh in the bag. Juveniles were also captured in a variety of other gears

including beam trawl, minnow traps, and seine nets; set locations for these gears were chosen based on prior reports of good gag catches or in a haphazard manner within structured habitats. In 2007, egressing juveniles were collected in channel nets in collaboration with commercial shrimp fishermen. Further details on postlarval and juvenile gag catch information can be found in Adamski et al. (2011).

Age and Growth Preparation.—Age (d) was estimated from postlarval and juvenile gag otoliths. Standard lengths (SL) of preserved specimens of postlarvae and juveniles were measured by caliper or ruler. Lapillar otoliths were removed and mounted on a slide and the clearest looking otolith (left or right) was chosen to be read per fish. Lapilli were used to age all fish with the assumption that otolith increments are deposited daily. Although the assumption of daily periodicity has not been validated for gag, other researchers have made this assumption for postlarval and juvenile gag (Keener et al. 1988, Rutten 1998, Fitzhugh et al. 2005) due to validation of daily increments in a variety of other reef fishes (Thorrold and Hare 2002). Sagittae are typically used in most aging studies, but they require more preparation than do lapilli and the latter have been used frequently to age postlarval and juvenile gag (Keener et al. 1988, Rutten 1998, Fitzhugh et al. 2005).

Lapilli from postlarvae were mounted on a slide in Depex mounting medium and read whole. Lapilli from juveniles were mounted on a slide in Depex, polished in the transverse plane due to their greater thickness, and read. Most juvenile otoliths could be read after polishing only one side while others had to be polished on both sides. Increments were counted in two separate steps for juveniles: counts were made inside the transition mark (IT) and outside of 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 postlarvae to benthic juveniles (Keener et al. 1988). Otoliths were read from photographs using an Olympus BX41 compound microscope at 200× for OT counts and 1000× 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 images for easier reading.

A single reader made a minimum of two counts for the IT and OT regions for one otolith (haphazardly chosen) per fish. If the two readings deviated <10%, their mean was computed and that was used as the estimate of duration within each otolith region. If the two counts deviated by >10%, a third reading was done. If any two of the three readings deviated by <10%, a mean of those two was computed and used; otherwise the otolith was not used for that region.

The PLD of juveniles was estimated by IT count (+3 d). The PLD of postlarvae was estimated as age at ingress (+3 d) although Keener et al. (1988) suggested that the transition mark may be present approximately 1.5 d before ingress. The 3-d adjustment is based on a 3-d discrepancy between lapilli and sagittae (Keener et al. 1988). As a check on this duration, we compared postlarval increment counts using sagittae and lapilli that were taken from the same fish (n = 48) and found the mean of the difference within individuals to be 3.25 d (SD 3.9). Sagittae were read from a transverse section.

The mean of the two increment counts for an individual for the IT (adjusted) and the OT regions were added together as an estimate of fish age since hatching. Hatch date was estimated by applying age to the date of collection. Fertilization was assumed to occur 3 d before hatch date given 2–3 d between fertilization and first increment formation in gag (Roberts and Schlieder 1983).

All postlarval gag (2007, 2008, and archived) were aged. Juvenile gag caught in 2007 and 2008 were subsampled and a minimum of two fish cm^{-1} (TL) increment was aged.

Data Analyses.—In 2007 and 2008, we examined the effect of collection month on fertilization dates for postlarval and juvenile gag using one-way ANOVA. Tukey's HSD was used as a post-hoc test. This test allowed us to determine if there were unrealistic (i.e., biased late or not observed in postlarvae) fertilization dates in juveniles that may have resulted from underaging of otoliths.

Fertilization dates obtained for postlarvae (2005 to 2008) in our study (North Carolina) were compared to fertilization dates obtained for postlarval gag collected in South Carolina by Keener et al. (1988, data from 1981 to 1983) using a Kolmogorov-Smirnov test. We did not use fertilization dates obtained from earlier years (<2005) in North Carolina because sampling in those years ended in April; postlarval gag from April collections have earlier fertilization dates relative to gag collected in May (see below).

Evidence of periodicity (e.g., lunar) in fertilization dates was evaluated using periodic regression (deBruyn and Meeuwig 2001) for postlarval gag (2007, 2008, and archived). This analysis was limited to postlarvae because age estimates for this stage were considered more reliable than those from juvenile gag (see below). Lunar days were defined as days from nearest full moon and were based on a 29-d lunar cycle [full moon = 0 and new moon = 14.5; lunar days were converted to radian angular equivalents (θ)]. For our periodic regression equations, cosθ describes peaks at either new or full moons while sinθ describes peaks at either the first or third quarter of a lunar cycle (location of peaks depends on sign of coefficient). Two peaks per lunar month can be described by the cos2θ or sin2θ term with locations of peaks (new, full, first, and third quarter) dependent on sign of coefficient. 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 predict the number of gag as a function of the above independent variables (various combinations of cosθ, sinθ, cos2θ, and sin2θ) assuming a Poisson distribution and a log link; analyses were done using R (R Development Core Team 2011) and Akaike's Information Criterion (AIC) was used for model selection. AICc (AIC with correction for finite sample sizes) values were used instead of AIC because of small sample size (Burnham and Anderson 2002).

To test if PLD had an effect on survival after settlement, one-way ANOVA was used to examine for an effect of collection month on PLD in 2007 and 2008. We hypothesized that fish with long PLDs would not survive and thus the distribution of PLDs would narrow and the mean be reduced. Factors that might influence the magnitude of PLD were also examined; relationships between PLD and fertilization date, ingress date, and SL were tested using Pearson product-moment correlation.

Apparent (i.e., size-selective mortality not accounted for) growth rates of juvenile gag were estimated during the summer months (June–August) of 2007 and 2008 using SL and estimated age (d) at collection. Postlarval gag were not included in the growth analyses because size did not vary with age at ingress ($P = 0.91$). Additionally, juveniles collected in fall (September–November) were excluded from this analysis because of suspected underaging (see below) of fish caught later in summer and to use data from the same collection periods in the between-year comparison. Apparent growth rates by year were estimated as the slope of a linear regression fitted to the size at age data; growth rates were compared using homogeneity of slopes test.

Results

In total, 206 otoliths were processed and used in the analyses. Ages were estimated from 56 juveniles and 19 postlarvae collected in 2007, from 53 juveniles and 14 postlarvae collected in 2008, and from 64 postlarvae from archived samples (1991–1998 and 2005–2006). Estimates of ages for the IT period were obtained for an additional three juvenile gag (one in 2007 and two in 2008), but increment counts in the OT region were not possible on these otoliths.

Estimates of fertilization dates of postlarval and juvenile gag were earlier for earlier months of collection in 2007 ($F_{5,69} = 32.335$, $P < 0.0001$; Fig. 2A) and 2008 ($F_{4,62} = 21.116$, $P < 0.0001$; Fig. 2B). In both years, fertilization dates in the early months of collections (with exception of April 2008; see below) were similar; however, fish collected from later months, after the ingress period was complete, often had estimated

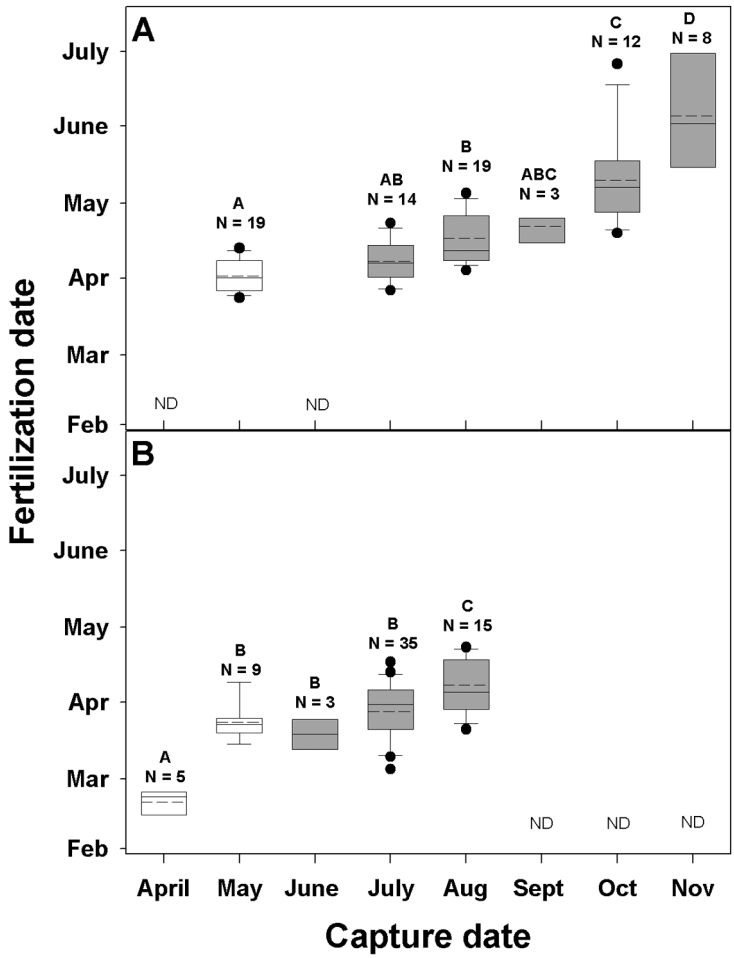

Figure 2. Estimates of fertilization dates for gag (*Mycteroperca microlepis*) by month of capture in (A) 2007 and (B) 2008. Open boxes represent fish caught as postlarvae and gray boxes represent fish caught as juveniles. Dashed line = mean; solid line = median; box boundaries = 25th percentile and 75th percentile; horizontal line = 10th and 90th percentile. Closed circles = outliers. ND = no data. Letters above monthly data denote results from multiple comparison tests.

fertilization dates that were significantly later (Tukey HSD: $P < 0.05$) than earlier collected fish (Fig. 2A,B). We conclude that many of the gag surviving to late summer and fall were spawned later or underaged; therefore, ages from juvenile gag were excluded from all further analyses with two exceptions: the PLD analysis (we assumed IT count was not influenced by underaging) and the growth rate analysis [estimates of apparent growth rate were limited to June–August because underaging was not as evident (i.e., many of these months did not differ in fertilization dates) and these months were consistently sampled across years]. Fertilization dates from postlarvae collected in April 2008 were from a spawning event in February 2008 (Fig. 2B); February fertilization dates were not represented in later collections.

Estimates of fertilization dates of postlarval gag (data from 2005 to 2008) collected in North Carolina ranged from February to April with the bulk in March

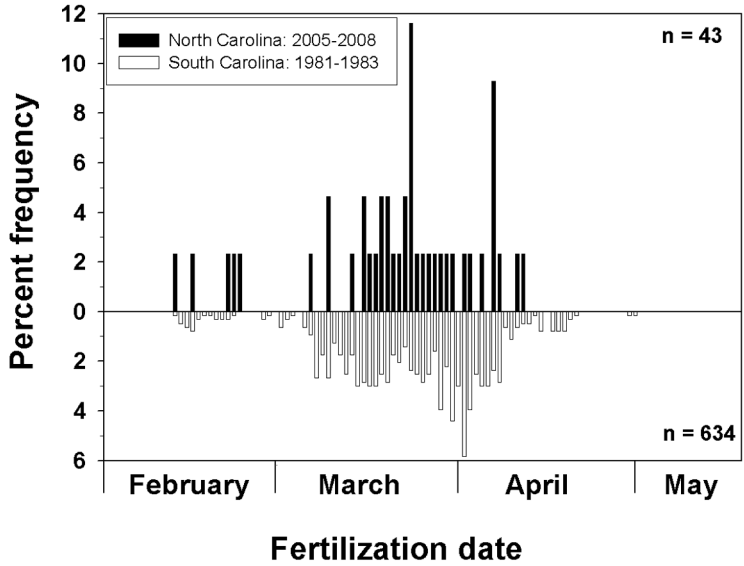

Figure 3. Percent frequency of fertilization dates estimated for postlarval gag (*Mycteroperca microlepis*) collected during ingress in North Carolina during 2005–2008 (filled bars; this study) and in South Carolina during 1981–1983 (open bars; Keener et al. 1988).

(Fig. 3). A similar distribution of fertilization dates was observed for postlarval gag collected in South Carolina (Keener et al. 1988; data from 1981 to 1983; K-S test: D = 0.144, $P > 0.10$; Fig. 3). Thus, timing of fertilization does not appear to differ between postlarvae collected in North Carolina and South Carolina and has not changed over an approximate 25-yr period.

Periodicity in fertilization dates was evident when counts of fertilization dates were plotted as lunar days (Fig. 4). Although there was evidence of spawning throughout the lunar cycle, fertilization dates were centered on first and third lunar quarters. Two different periodic regression models supported this conclusion (Table 1). Model 1 had slightly higher weight and predicted a higher number of spawnings in the third quarter than the first quarter while model 2 predicted comparable spawning frequency for the two quarters (Table 1; Fig. 4).

Mean PLD in gag differed little between years (44 and 45 d for 2007 and 2008, respectively). PLD did not vary with collection month in either year (2007: $F_{5, 70} = 0.429$, $P = 0.827$, Fig. 5A; or 2008: $F_{4, 64} = 1.445$, $P = 0.229$, Fig. 5B). Thus, there was no decrease in PLDs over time, which would be evidence for selective loss of gag with relatively long PLDs.

Postlarval gag from later fertilization dates had shorter PLDs (Fig. 6A; $r = -0.41$, $P < 0.001$). However, there was no relationship between PLD and postlarval ingress date (Fig. 6B, $P = 0.71$) or postlarval size (Fig. 6C; $P = 0.25$). PLDs of postlarvae were variable at ingress, but size was not [Fig. 6C; 2007: PLD (age) range 32–52 d, SL range 12–17 mm; 2008: PLD range 34–61 d, SL range 13–18 mm].

Apparent growth rates of juvenile gag were >1 mm SL d^{-1} (2007 = 1.4 mm d^{-1}; 2008 = 1.3 mm d^{-1}) during summer and these annual estimates of growth rates (slopes) did not differ between years (homogeneity of slopes test: $F_{1,85} = 1.413$, $P = 0.238$; Fig. 7).

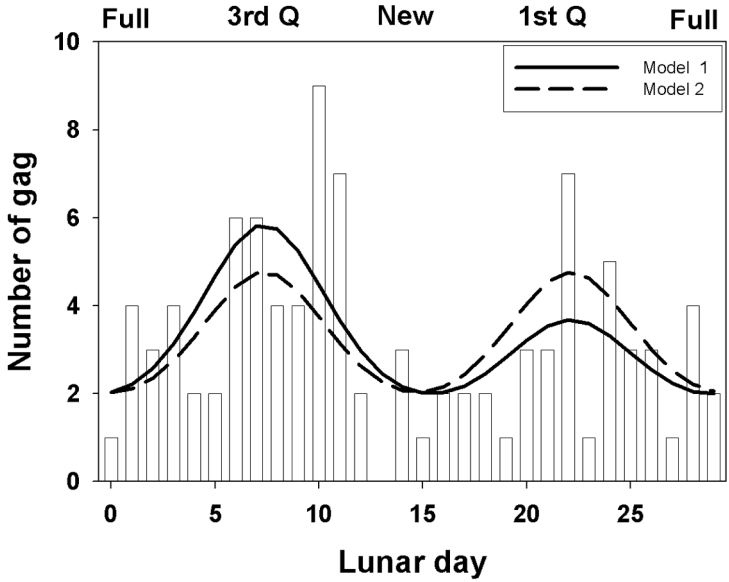

Figure 4. Frequency of fertilization dates (bars) across lunar cycle based on otolith increments from postlarval gag (*Mycteroperca microlepis*) caught at ingress from 1991 to 2008. The predicted frequencies (solid line: model 1; dashed line: model 2) from the best predictive models (Table 1) are plotted with the observed (bars) data. The equation for model 1 is: $\ln(\text{gag}) = 1.122 + 0.231*\sin(\Theta) - 0.413*\cos(2\Theta)$, and for model 2 is: $\ln(\text{gag}) = 1.135 - 0.426*\cos(2\Theta)$.

Discussion

Fitzhugh et al. (2005) collected juvenile gag from multiple locations along a latitudinal gradient on the west coast of Florida. They found regional differences in fertilization and settlement dates of gag among sampling locations. Given this latitudinal effect, we investigated whether the timing of spawning, which results in postlarval gag ingress into North Carolina (northern limit of their range), differed from a previous otolith-derived fertilization date study conducted in South Carolina (Keener et al. 1988). We found fertilization to occur from February to April with a peak from late March to early April. This is consistent with Keener et al.'s (1988) findings and from histological analyses of gonads in the SEUS (Collins et al. 1987). Additionally, there is overlap in spawning times (February–April) between SEUS and Gulf of Mexico (McErlean 1963, Koenig et al. 1996, Collins et al. 1998), though peak spawning in SEUS is more similar to that estimated for aged recruits captured in northern Gulf of Mexico estuaries (Koenig and Coleman 1998, Fitzhugh et al. 2005) compared to estimated spawning dates for recruited gag caught in southwest Florida estuaries (Fitzhugh et al. 2005). Thus along the SEUS coast there do not appear to be regional differences in fertilization and settlement dates of gag.

Based on ages of postlarvae collected in North Carolina, gag spawning in 2007 and 2008 occurred throughout the lunar cycle but peaked around first and third quarter moons. Keener et al. (1988) also found evidence for spawning throughout the lunar cycle from South Carolina collected postlarvae, but peaks in spawning were closer to new and full moons. Periodicity in spawning within a lunar cycle has been

Table 1. Periodic regression models describing numbers of gag (*Mycteroperca microlepis*) by fertilization date throughout a lunar cycle based on otolith age estimates from gag postlarvae (n = 97). Postlarvae were caught at Pivers Island, North Carolina, from 1991 to 2008. Gag = null is an intercept only model. Models are ranked by ΔAICc and Akaike model weights (w). Models with ΔAICc values <2 have substantial support (Burnham and Anderson 2002). Θ = lunar day in radian angular equivalents.

ID	Model	AICc	ΔAICc	w
1	gag = sin(Θ) + cos(2Θ)	119.64	0.00	0.57
2	gag = cos(2Θ)	120.50	0.86	0.37
3	gag = sin(Θ)	125.48	5.84	0.03
4	gag = null	127.05	7.41	0.01
5	gag = sin(2Θ)	127.39	7.75	0.01
6	gag = cos(Θ)	129.15	9.51	0.01
7	gag = cos(Θ) + sin(2Θ)	129.55	9.91	0.00

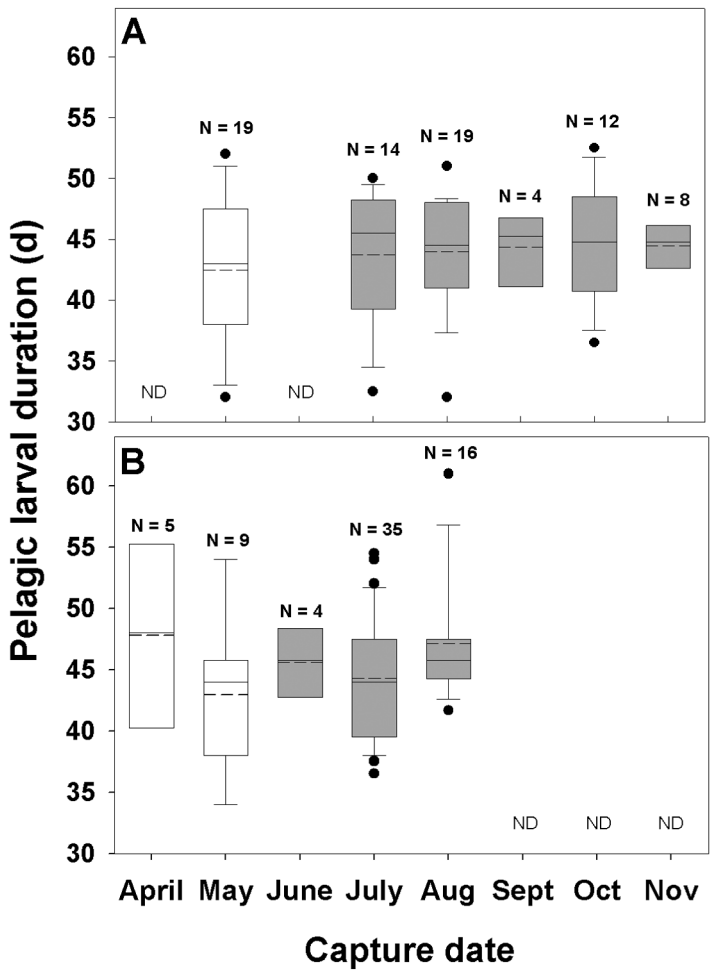

Figure 5. Estimates of pelagic larval durations for gag (*Mycteroperca microlepis*) by month of capture based on otolith increments from (A) 2007 and (B) 2008. Open boxes represent fish caught as postlarvae in bridgenet and gray boxes represent fish caught as juveniles. Dashed line = mean; solid line = median; box boundaries = 25th percentile and 75th percentile; horizontal line = 10th and 90th percentile. Closed circles = outliers. ND = no data.

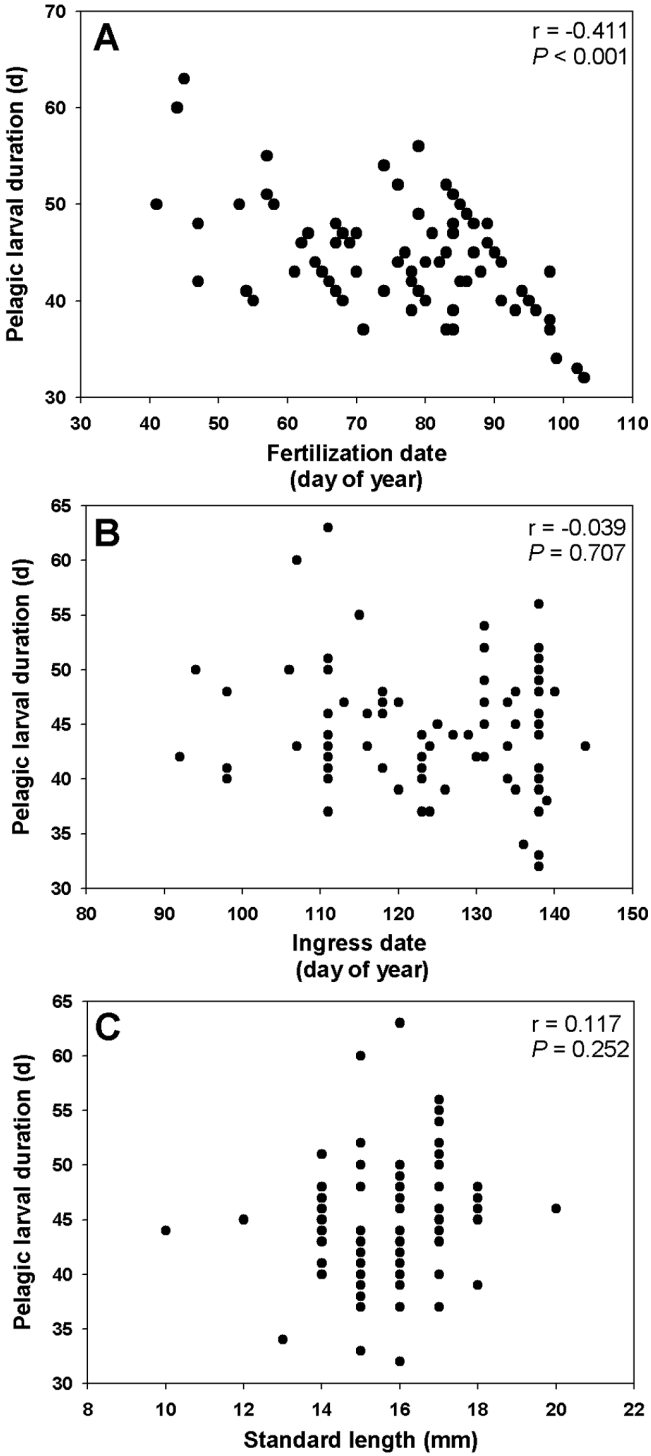

Figure 6. Relationships between pelagic larval duration of postlarval gag (*Mycteroperca microlepis*) from 1991 to 2008 and (A) fertilization date, (B) ingress date, and (C) standard length.

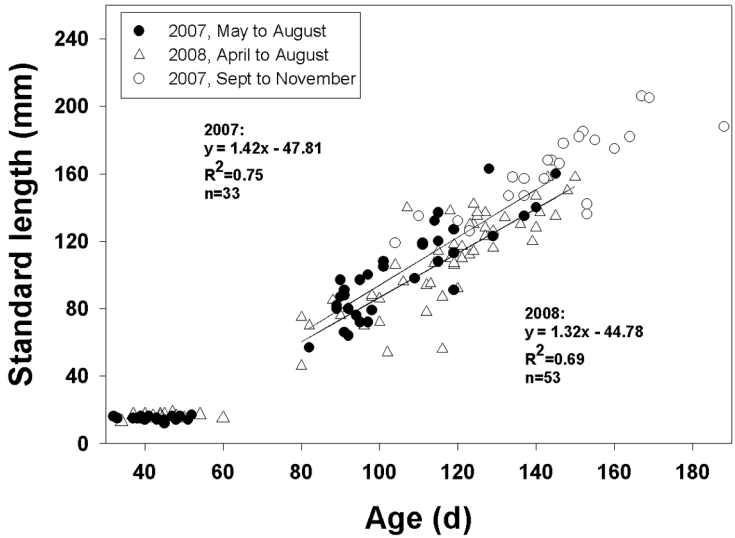

Figure 7. Relationships between standard lengths (mm) of postlarval or juvenile gag (*Mycteroperca microlepis*) and estimated age at collection based on otolith increments for 2007 and 2008. Lines represent linear regression fits (details on graph) for the June to August collected juvenile gag. The regression analysis did not include postlarvae collected in April or May (less than or equal to approximately 20 mm SL) because we were not interested in estimating growth of ingressing postlarvae. Nor did the regression include juveniles collected after August because of concerns that these individuals were underaged (see text).

found for other epinephelids; for example, orange-spotted grouper, *Epinephelus coioides* (Hamilton, 1822), had peak gonad weight at full and new moons with spawning thought to occur after these peaks (Grandcourt et al. 2009). The reason behind the difference in lunar periodicity between the Keener et al.'s (1988; new and full moon) and our results (first and third quarter moon) is unclear and could reflect real differences in spawning times, differences in survival or ingress of larvae, or systematic differences in aging between groups.

Gag form annual site-specific spawning aggregations, which results in high catchability (Gilmore and Jones 1992, McGovern et al. 1998). Because of this, spawning closures are thought to be effective at reducing fishing mortality rates; these temporal closures were recently lengthened from March–April to January–April in SEUS (SAFMC 2009). Only indirect methods have been used to assess spawning activity of gag because spawning events have never been observed (Koenig et al. 1996). Our information on spawning periods provides further justification for the recent inclusion of January and February to the spawning season closure in the SEUS.

Our estimates of gag fertilization dates shifted to later dates beginning with August collections; we believe at least part of this shift is due to underaging in older juvenile gag as the later fertilization dates were not represented in the last groups of ingressing gag or recently settled juvenile gag. Counting otolith increments in older, larger juvenile fish is difficult because the outer increments often coalesce (Ahrenholz et al. 1995). Rutten (1998) back-calculated fertilization and ingress dates using lapilli from juvenile gag caught from 26 August to 29 September, 1997, in New River, North Carolina. 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 approximately 20–30 d later when compared to estimated fertilization dates from younger, smaller gag and observed for postlarval ingress in North Carolina and South Carolina (Keener et al. 1988, present study). Given the evidence for underaging in our study and inferred from Rutten's (1998) study, we conclude that daily ages from juvenile gag collected in late summer may be biased high. Although the bulk of the shift in fertilization dates is likely due to underaging, some of this shift could be due to ontogenetic habitat shifts (see Ross and Moser 1995) of older/larger juveniles out of the grass beds; that is, if earlier spawned gag are no longer available to the trawl because of emigration then the remaining smaller (and potentially younger) gag might have later fertilization dates.

Estimates of PLDs for gag recruiting to estuaries of Florida and the Carolinas are consistently approximately 45 d (Keener et al. 1988, Rutten 1998, Fitzhugh et al. 2005, present study). Gag have a moderately long PLD when compared to other reef fishes (Victor 1986b, Cowen and Sponaugle 1997) and even with other *Mycteroperca* spp. in the eastern Pacific (approximately 24 d; Benjamin Victor, Ocean Science Foundation, pers comm). 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, *Lutjanus griseus* (Linnaeus, 1782), a reef species that shares a similar life history with gag, caught at the Pivers Island bridgenet site in warmer summer months had a mean PLD of 27 d, yet the nearest known spawning grounds for gray snappers are hundreds of kilometers to the south (Tzeng et al. 2003). The trend toward shorter PLDs in postlarval gag spawned later in the season is likely due to water temperature (effects on larval development) or primary productivity (Keener et al. 1988); the size at ingress may be developmentally controlled and development rate is faster in warmer months leading to shorter PLDs. Fitzhugh et al. (2005) also found a negative relationship between PLDs and fertilization date in gag in 2 of 4 yrs based on juveniles collected in eastern Gulf of Mexico and speculated faster growth or transport were responsible.

Gag had high variability in PLDs while the size at ingress was relatively consistent. Thus, larval growth rates were variable. For a variety of reef fishes, Wellington and Victor (1992) found faster growing reef fishes spent less time in the plankton than slower growing fish. Cowen (1991) determined post-settlement growth was unaffected by the presence of a slow growth pre-settlement period in California sheephead, *Semicossyphus pulcher* (Ayres, 1854), suggesting long PLDs were not detrimental to post-settlement survival. We tested the hypothesis that gag with longer PLDs have lower post-settlement survival. We did not have estimates of survival to test this hypothesis directly; instead, we examined PLDs of juvenile fish collected monthly after settlement to test whether fish with longer PLDs were selectively removed over time (see Searcy and Sponaugle 2001). We found no evidence for this hypothesis; PLDs observed in postlarval gag were also observed in late-stage juveniles collected up to 180 d after settlement.

Gag residing in any given estuary in the eastern and western Gulf of Mexico (Koenig and Coleman 1998, Reńan et al. 2006) and in North Carolina (Ross and Moser 1995) have been described as a single cohort (i.e., produced from one discrete spawning period). The consistent fertilization dates over several months and the low variability in size at capture date (Adamski et al. 2011) are consistent with these prior studies. We conclude that gag recruiting to North Carolina estuaries are produced by a single cohort spawned during February–April. Interestingly, the February

fertilization dates determined from the pulse of postlarval gag caught in April 2008 were not observed in subsequent collections. We expected to see February-spawned gag as settled juveniles because 11% of the 97 postlarvae examined in our study came from February fertilization. February-spawned gag may not survive once settled but further work is needed in multiple years and other estuaries to determine the consistency of this pattern.

Apparent growth rate estimates using size and otolith age were approximiately 1.4 mm SL d^{-1} and are similar to growth rates estimated using size and capture date in North Carolina (Ross and Moser 1995, Adamski et al. 2011). Similarly, juvenile gag collected from oyster reef habitats in South Carolina and aged with otoliths grew at approximately 1.3 mm SL d^{-1} (Mullaney and Gale 1996), but there was a wide range in otolith age-derived growth rates from different Florida estuaries (1.07 to 1.85 mm SL d^{-1}; Strelcheck et al. 2003). Juvenile gag growth rates are similar to other juvenile estuarine fish that have diets dominated by fish prey (see Juanes and Conover 1995 for review); fish and large shrimp prey become increasingly important as gag grow during summer (Ross and Moser 1995, Mullaney and Gale 1996).

We found that postlarval and juvenile gag collected in North Carolina shared early life history traits with those described for South Carolina and western 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 and appears to be associated with the lunar cycle. Pelagic larval duration in all three regions averages approximately 43 d; in North Carolina, there was no evidence for an influence of PLD on survival to the late juvenile stage. We were unable to confidently age juvenile gag in late summer and fall months due to underaging, which most likely resulted from coalescing of outer increments. Juvenile gag grow rapidly while residing in North Carolina seagrass beds and this habitat should be protected for its nursery function (Deaton et al. 2010). The results of this study support the South Atlantic Fishery Management Council's inclusion of January and February to the original March and April spawning season closure (SAFMC 2009).

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