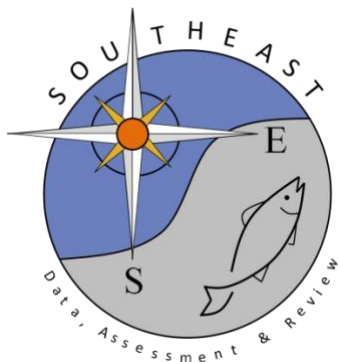


# Is the BOFFF (Big Old Fat Fecund Females) hypothesis applicable to Gulf of Mexico greater amberjack?

Debra J. Murie, Daryl C. Parkyn, and Andrew Fischer

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# MARFIN PROJECT FINAL REPORT

## **IS THE BOFFF (BIG OLD FAT FECUND FEMALES) HYPOTHESIS APPLICABLE TO GULF OF MEXICO GREATER AMBERJACK?**

Submitted by

Debra J. Murie and Daryl C. Parkyn  
Fisheries and Aquatic Sciences Program  
School of Forest Resources and Conservation  
University of Florida, Gainesville, FL 32653

Andrew Fischer  
Louisiana Department of Fisheries and Wildlife  
2000 Quail Drive, Baton Rouge, LA 70808

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## Executive Summary

Greater Amberjack in the Gulf of Mexico are considered to be overfished and for overfishing to be occurring. Increased regulatory measures have not had the expected outcome of rebuilding the stock. There has therefore been an increased need for better reproductive information, release mortality, and potentially alternative management strategies. The goal of this study was to explore whether **Bigger, Older, Fatter, and more Fecund Females (BOFFFs)** contribute disproportionately more to the reproductive potential of the spawning stock of greater amberjack in the Gulf of Mexico; and if so, would an alternative management plan based on conserving the reproductive potential of the BOFFFs using a harvest-size slot limit be more efficacious for rebuilding the stock compared to using a minimum size limit?

To meet this goal, our first major objective was to quantify the bigger, older, and more fecund female factor (the BOFFF factor) in greater amberjack. We did this by sampling 1,024 fish off the coast of Louisiana, USA, as coastal waters and rigs are known to hold spawning aggregations of Greater Amberjack. We sampled fish during their punitive spawning season, from January through June in 2016 and 2017; of these, 569 were females and 383 were males. Females were the focus of this study and hereafter the summary relates only to females. Greater Amberjack ranged in size from 340-1373 mm fork length (FL) and 640 g to 36.6 kg. Age frequencies ranged from 1 to 9 years, with the majority of fish in age classes 2-5. Fish were sampled with the goal of estimating the duration and peak of the spawning season, spawning fraction of females and hence the interval between spawning batches, age- and size-specific batch fecundity, and to re-evaluate age- and length-based sexual maturity in relation to histological spawning biomarkers and reproductive staging.

Peak spawning season and duration were estimated using both histological reproductive staging and gross morphology of the ovaries. Very few hydrated females were observed in the study (4 out of 569 females), which is very similar to Harris et al. (2007) in their study of Greater Amberjack spawning in south Florida. Females in an actively spawning subphase were therefore identified by oocytes in final maturation, either with germinal vesicle migration (GV), yolk coalescence/germinal vesicle breakdown (YC/GVBD), or newly collapsed post-ovulatory follicles (POFs). Based on histological study of individual oocyte stages in the ovaries of mature females, the peak spawning season for Greater Amberjack in the northern Gulf of Mexico was in March, April, and May.

The peak spawning season was also clarified by using an analysis of reproductive phases, which designates mature females as developing (not spawning capable at the time of collection), spawning capable (will spawn in the current spawning season), and post-spawning females (regressing and regenerating). A large percentage of females (~80-90%) were in the spawning capable phase by March and continued into May.

Further, the gonadosomatic index ( $GSI = \text{ovary weight} / \text{total weight}$ ) for mature female greater amberjack was also different among spawning months, and highest in March, April and May (2.8-3.4% of female body weight), indicating peak spawning. Females assigned an immature status had GSI values  $<0.5\%$  of their body weight, confirming their non-participation in the current spawning season.

The duration of the spawning season was estimated as ~76-77 days based on the observation of females in the actively spawning subphase as early as mid-March and as late as 1 June. Harris et al. (2007) similarly estimated the spawning season to be ~73 days off of South Florida, based on the criterion of females being in final oocyte maturation.

The spawning fraction of mature females in January and February was zero because no females were actively spawning even though mature females collected were spawning capable (V3 oocytes present). The spawning fraction was fairly consistent over the peak spawning months of March, April, and May (0.20-0.25) and then markedly decreased to 0.083 in June. However, the decrease in spawning fraction is in concert with the decrease in spawning activity observed in the histological and GSI analyses. Harris et al. (2007) observed a similar spawning fraction for greater amberjack in the South Atlantic, reporting an average of 0.227.

Based on the spawning fraction, the interval between spawning batches of eggs was consistent among the months of March through May, and then increased markedly as the spawning fraction decreased in June. On average, greater amberjack were capable of spawning every 4.5 days through the months of March to May, with the interval increasing to ~12 days between spawning events in June. Similarly, Harris et al.'s (2007) average spawning fraction corresponded to a spawning interval of ~5 days.

The relationship between batch fecundity and female fork length was positive and significant ( $r^2 = 0.58$ ). Overlaying the batch fecundity regression reported in Harris et al. (2007) showed that greater amberjack in the South Atlantic have a greater batch fecundity in relation to their length compared to greater amberjack in Louisiana waters. Batch fecundity as a function of female weight was also significant, linear, and positive ( $r^2=0.72$ ); whereas the relationships with fish age was more variable but still significantly positive ( $r^2=0.33$ ).

Sexual maturity was estimated based on three criteria: 1) females designated as mature based on being in the developing phase and beyond (cortical alveolar oocytes, CA, and beyond), with a  $L_{50\%}$  of ~827 mm FL; 2) females designated as mature based on having early stage vitellogenic oocytes (V1 and V2, and beyond) but not CA oocytes, with a  $L_{50\%}$  of ~861 mm FL; and 3) females designated as mature based on reaching the spawning capable phase with late vitellogenic oocytes (V3) and beyond, with a  $L_{50\%}$  of ~873 mm FL. The largest difference was the inclusion of females that had oocytes in the cortical alveolar (CA) stage, which was based on them being physiologically mature but not ready to spawn at the time of their collection (i.e., developing ovaries in potential preparation for the upcoming spawning season).

The increase in ovary weight as a function of female size over the months of Jan to June lent support to both the size at sexually maturity and the peak spawning season. Ovary weight did not increase significantly until females reached ~800-850 mm FL, and increased significantly in March, April and May.

Our second major goal was to model the “fatter” factor in the BOFFFs using the relationship between the caloric density in their white muscle, liver, and ovaries fish typically use lipids as a source of energy, storing it in their larger white muscle tissues or liver, and transferring it to the ovaries for use in oocyte development and other spawning activities (i.e., migration). Overall,

caloric density of muscle tissue (cal/g) only showed a slight positive increase in relation to female size in all months collected. More definitively, females in February (and to a lesser extent January) prior to the start of peak spawning, had higher levels of energy reserves in their liver tissue than other months and the liver energy density increased with female size. Liver energy densities in the peak spawning months of April and May were low and comparable to that of June in the post-spawning period. This indicated that females were most likely transferring energy from their lipid-rich livers to their ovaries starting in March. Caloric density of ovarian tissue showed a significant increase in relation to female size during the months of March-May. In addition, females that had increased ovary weight in March, April and May (females  $\sim > 860$  mm FL) had ovaries with higher caloric density than similar-sized females in January, February and June. This is presumably due to the presence of lipids (i.e., high energy density) in the ovaries (oocytes) of females ready to spawn. This was supported by the decrease in liver energy density during the same period. There appears to be a size threshold for females ( $\sim 800$ - $850$  mm FL) in having large ovaries with high energy density during the peak spawning months and this may be directly related to their ability to spawn (or not, if energy density is too low).

Our third major goal was to use an age/size-structured population model to evaluate the use of a minimum size limit versus a harvest-size-slot (harvest slot) limit aimed at reducing fishing mortality on BOFFFs, and evaluate the effects of these harvest regulations on the number of large fish harvested relative to fisheries yield. We explored the potential of a model derived from Gwinn et al. (2013) to examine the effect of the minimum size limits and Harvest Slot width on the possible proportion of fish available for harvest and the proportion of large-sized fish available. To test the potential for this method of management, we initially examined three minimum length limits: 28' (711 mm), 34" (863 mm), and 36" (914.4 mm). Under current regulations, FL = 34" (863 mm) and 36" (914 mm) are regulatory length minimums for recreational and commercial fisheries, respectively, and fish above these length minimums are open to harvest. As a proof of concept, regardless of minimum size, as the slot size widens, the potential number of fish harvested increases rapidly but a greater minimum size does provide some reduction in harvest and a bit more protection to larger reproductive fish. However, a smaller minimum size coupled with a narrow slot size, limiting retention, may lessen the probability of retention of large reproductive fish, increasing their prevalence in the reproductive pool relative to a minimum size limit only scenario. A slot limit may be a means to reduce or end retention of BOFFFs, because the majority of Greater Amberjack over 1 m are females. Given the low hooking mortality rate of Greater Amberjack in vertical-line fisheries, this may be an alternative strategy that can be implemented. This proof of concept in the use of a harvest slot for Greater Amberjack could be explored more fully with a stock assessment approach to simulate alternative slot sizes in relation to rebuilding spawning stock biomass since Greater Amberjack are overfished and experiencing overfishing.

## INTRODUCTION

Greater amberjack is widely distributed throughout warm temperate and tropical waters and is an important recreational and commercial fishery in the Gulf of Mexico (Burch 1979; Manooch and Potts 1997). The recreational catch for amberjack in the Gulf of Mexico has historically exceeded commercial hand-line/longline and headboat landings on a Gulf-wide basis (Berry and Burch 1977; Manooch and Potts 1997; Cummings and McClellan 2000; SEDAR 2006, 2014). Landings from the west coast of Florida and Louisiana have dominated recreational (private and charter) and commercial (handline) catches of amberjack in the Gulf (SEDAR 2006, 2014).

Gulf of Mexico greater amberjack have been regulated since 1990 with a daily bag and a minimum size limit [28 inch fork length (FL)]. Starting in the 1990s, regulations were steadily increased on greater amberjack in the Gulf, including decreasing the daily bag limit to one fish, imposing a zero bag limit for captain and crew of for-hire vessels, increasing the minimum size limit (currently 34 inches FL), enacting a closed season for commercial fisheries, and setting quotas (ACLs) for both the recreational and commercial fisheries that result in closure of the fisheries once the ACLs have been reached (SEDAR 2014). Despite these ramped-up fishing regulations, the most recent stock assessment update for Gulf greater amberjack in 2016 (SEDAR 2016) concluded that Gulf greater amberjack are overfished and underdoing overfishing. Greater amberjack have been under a rebuilding plan since 2003 with the purpose of ending overfishing and restoring the stock to the biomass level ( $B_{MSY}$ ) capable of producing maximum sustainable yield (MSY) on a continuing basis. With the latest full stock assessment (SEDAR 2014), it became apparent that the stock has not met the 10-year rebuilding plan that ended in 2012; further regulations will therefore need to be implemented to allow rebuilding of the stock.

## BACKGROUND INFORMATION AND IDENTIFICATION OF THE PROBLEM

The most recent stock assessment for Gulf of Mexico (Gulf) greater amberjack was based on a statistical catch-at-age model configured using Stock Synthesis (Methot 2013, cited in SEDAR 2014), which was the preferred model in the 2014 assessment compared to the surplus production model used for continuity with the previous assessment and update (SEDAR 2006, SEDAR 2010). Reproductive potential of female greater amberjack in the Gulf stock has typically been captured in assessments by using female body weight as a proxy for fecundity. This was the case in the statistical catch-at-age model used in the most recent stock assessment (SEDAR 2014), where “The fecundity schedule was assumed directly proportional to female weight in the assessment model.” (SEDAR 2014 SAR, page 17). This has been deemed a reasonable assumption because of an expected proportional relationship between fecundity and female body weight (Hunter et al. 1985), with the extension that female spawning biomass used in the stock assessments is a proxy for total egg production (Fitzhugh et al. 2012). Previous stock assessments for Gulf greater amberjack have also had to rely on borrowing reproductive data from a study on Atlantic (Florida Keys) greater amberjack (Harris et al. 2007). With knowledge of fecundity as a function of female weight from Harris et al. (2007) based on the Atlantic stock of greater amberjack, female weight can be converted to total egg production (e.g., eggs/kg female weight) (SEDAR 2104). The underlying assumption in these stock assessments is that spawning duration and spawning frequency are invariant across age or size (Fitzhugh et al.

2012). This means that regardless of whether the female is spawning for the first time as a 2 or 3 year old fish or for the fifth time as a 7 year old fish, both females are factored into the stock assessment as producing the same number of eggs on a per kg body weight basis during the spawning season. There is a growing body of evidence to suggest that this is not a valid assumption for many fish species and that, in general, BOFFFs contribute disproportionately more because spawning duration and spawning frequency increase with increasing fish age and size (Claramunt et al. 2007; Lowerre-Barbieri et al. 2011; Fitzhugh et al. 2012).

Attributing enhanced reproductive potential to **Big, Old, Fat, Fecund, Females** in fishes is not a new concept and has aptly been named the **BOFFF** hypothesis (also BOFFFF, in reference to Fish). The BOFFF hypothesis has generally been associated with the work of Berkeley et al.'s (2004 a,b) research on Pacific rockfishes (*Sebastes* spp). Berkeley recognized that very old, large, fecund female rockfish that were in good "condition" (i.e., plump for their length, or the **Fat** factor) produced many more larvae (rockfish are live bearers) than on a proportional basis on their weight alone (Berkeley et al. 2004a,b; Bobko and Berkeley 2004). In addition, they found that the larvae of these BOFFFs survived better than larvae of smaller females, either because they were slightly larger at birth or had been provisioned with greater lipid (fat) reserves via the maternal contribution (Berkeley et al. 2004a,b).

Integration of maternal effects into fisheries stock assessments is novel, however, and most assessments do not yet take into account the added reproductive potential of these BOFFFs, above and beyond fecundity based on weight alone (Hixon et al. 2013). There is a growing recognition, however, that these effects need to be incorporated in the determination of reproductive potential in stock assessments due to the potential for estimates of stock productivity to be biased (Venturelli et al. 2009; Fitzhugh et al. 2012; Hixon et al. 2013). Models based on per-recruit analyses of spawning potential ratio and reproductive value showed substantial sensitivity to age-dependent spawning frequency (i.e., the number of batches spawned each spawning season) (Fitzhugh et al. 2012). In particular, Fitzhugh et al. (2012) demonstrated that stock assessment models would tend to overestimate biological reference points (e.g., spawning potential ratio) if they assumed that the annual number of batches was age invariant when in fact they were shown to increase with fish age. Modeling studies on Pacific Ocean perch (*Sebastes alutus*) and Pacific cod (*Gadus macrocephalus*) also have shown that stock productivity is overestimated if maternal effects are not incorporated when in fact they exist in the population, especially at low stock sizes (Spencer et al. 2014). Venturelli et al. (2009), in their meta-analysis of long-lived north temperate and Arctic fish species, also demonstrated that stocks with a full age structure had higher reproductive rates than stocks with a truncated age structure; this was independent of spawning stock biomass.

Age-dependent spawning duration and frequency are particularly difficult to estimate in many fishes in the southeastern United States because they tend to spawn over an extended period of time while releasing multiple batches of eggs during any one spawning season (Hunter et al. 1985). In an extensive literature review and meta-analysis, Fitzhugh et al. (2012) documented that in studies where spawning duration and frequency were examined, 82% and 62% of the fish species spawned over a longer duration, and more frequently, with increasing age or size, respectively. The reproductive potential of the stock may therefore be dependent not only on the total cumulative biomass of the reproductively active females, but also on the age and size of the

females comprising the spawning stock (i.e., 1 kg of a 3-year old female is not equivalent to 1 kg of a 7 year old female).

Greater amberjack in the Gulf of Mexico are no exception to the many marine fishes in the southeastern United States that have a pattern of asynchronous, indeterminate, batch spawning (Murie and Parkyn 2008). Functionally, this means: 1) individual fish spawn at different times within the spawning season (asynchronous) (Lowerre-Barbieri et al. 2011); 2) females continuously recruit new oocytes during the spawning season (i.e., oocytes in different stages of development occur together in the ovary) (indeterminate); and 3) females develop and release multiple “batches” of the mature eggs over a single spawning season (Hunter and Macewicz 1985). Therefore, in order to estimate the total annual potential fecundity of a female greater amberjack, one needs to know the time period over which she spawns (spawning season duration), the frequency with which she spawns over the spawning season (or the average number of days between batches), and her batch fecundity (i.e., the number of eggs she releases each time she spawns a batch of eggs), on an age/size-specific basis. Currently, spawning duration, frequency, batch fecundity, and hence total annual potential fecundity by age/size of female are unknown for greater amberjack in the Gulf of Mexico.

Although the BOFFF hypothesis has been applied mostly in very long-lived marine fishes, such as Pacific rockfishes, it can also be relevant to even moderately-lived fishes, such as typical snappers and groupers in the southeastern United States. A case in point, Fitzhugh et al. (2012) recently demonstrated that the spawning fraction (and hence the spawning frequency) in female red snapper (*Lutjanus campechanus*) in the southeastern United States increased substantially with age. Fitzhugh et al. (2012) summarized known fish species that increased in spawning duration and spawning frequency with age and size and many of these fishes were relatively short-lived species, such as clupeiforms (herrings, anchovies, sardines), sciaenids (croakers), scombrids (chub mackerel), and carangids (jack mackerel). In addition, Lowerre-Barbieri et al. (2009) demonstrated that spotted seatrout (*Cynoscion nebulosus*) have increased spawning duration and increased spawning frequency as their age and size increase, with most fish between the ages of 1 and 7 years old on the spawning grounds (Lowerre-Barbieri et al. 2011). Indeed, the BOFFF hypothesis may be applicable to any fish species that demonstrates variability in reproductive parameters with age/size. So although most of the greater amberjack landed in the Gulf fisheries are only between 3 and 7 years old (SEDAR 2014), they are an ideal species in which to test the BOFFF hypothesis because they grow extremely quickly to a large size, spawn over a relatively lengthy season (January to June; Murie and Parkyn 2008), and have a skewed sex ratio for fish > 1 m FL in favor of females (1 male to 2.3 females) (Murie and Parkyn 2008; Smith et al. 2014). BOFFFs may therefore contribute disproportionately to the overall reproductive potential of the spawning stock in the Gulf.

Over-exploited stocks, as well as many sustainably harvested stocks, result in a shift in the age structure of the stock to younger (and smaller) individuals (i.e., juvenation) (Berkeley et al. 2004b). One consequence of this truncation of the age structure is the potential for increased risk to the stock since younger and smaller females are predicted to spawn over a shorter duration and/or produce fewer batches of eggs (“variance dampening”). In particular, this makes the stock more susceptible to environmental changes or perturbations that occur during the time frame of the shortened spawning season of these younger females. Consequently, it can also



affect the survival of the eggs and larvae they produce (e.g., temperature fluctuations, variability in plankton abundance for food, etc.). Variability in the timing and duration of spawning by females of varying ages, and their associated “peak” in spawning, may in part protect the stock by “spreading-out” the risk associated with spawning and hence survival of eggs or larvae. This can be seen with older and larger females, that are predicted to spawn over a longer duration and more frequently, being the buffer against human (i.e., over-exploitation) and environmental (i.e., oil spills) perturbations (Vallin and Nissling 2000; Hsieh et al. 2010; Berkeley 2006). Shindler et al. (2010) found that the risk of having an entire cohort of salmon exposed to suboptimal environmental conditions was reduced by having a diversity of age structures in the population because the population dynamics of the stock were slightly variable with respect timing (not 100% synchronous). Even in rockfishes that give birth to their live young (parturition) all at once, older females have been reported to parturate earlier in the season compared to younger and smaller females (Eldridge et al. 1991; Nichol and Pikitch 1994; Berkeley and Markle 1999). In black rockfish (*Sebastes melanops*), parturition during certain time periods results in higher survival of the larvae (Berkeley and Markle 1999). The natural variability in the timing and the quality of reproductive events in BOFFFs in response to environmental variability may reduce the recruitment variability seen at the stock level.

In addition to an increased reproductive potential related to the **quantity** of eggs produced in BOFFFs, there is also a potential difference in the **quality** of eggs produced by females of different ages/sizes. In some fish species, egg size increases as a function of female body size (e.g., haddock *Melanogrammus aeglefinus* and Atlantic cod; Hislop 1988, Kjesbu 1989). This relationship is important because smaller eggs have reduced survival. Equally important, egg quality may be lower for first-time spawners compared to fish that are experienced spawners (e.g., Atlantic cod; Trippel 1998). In the live-bearing blue rockfish (*S. mystinus*) and yellowtail rockfish (*S. flavidus*) the oil globule volume in the eggs was larger as fish increased in age/size (Sogard et al. 2008). Larson (1991) also observed that condition and lipid reserves increased disproportionately in female rockfish with increasing size and age, which indicated that the BOFFFs had greater energy reserves that they could allocate to reproduction compared to younger and smaller females. Quality of the eggs may therefore be related to their size as well as their supply of nutrients. Increased energy allocation per egg or larvae by older females is most often linked to increased larval survival because the larvae have an extended energy source that they can use prior to encountering a suitable source of food, reducing starvation events (Berkeley et al. 2004a; Sogard et al. 2008).

If BOFFFs can disproportionately enhance the reproductive potential of a stock, as well as potentially decrease the risk associated with environmental changes and perturbations and overexploitation, then should we consider a management strategy that conserves the BOFFFs rather than targets them? Under what scenarios should we consider a harvest slot limit versus maintaining the status quo with a minimum size limit? A harvest slot limit constrains the take of fish to an intermediate size range, which allows the younger and smaller fish to attain sexual maturity and “spawn once” prior to capture while also decreasing the fishing mortality on the very large reproductively active fish. In the case of greater amberjack, these very large reproductively active fish are BOFFFs in most scenarios because of the female skew in the sex ratio for fish > 1 m FL (Smith et al. 2014).

The appropriateness of using minimum size limits to regulate fisheries is being challenged on several fronts, ranging from genetic considerations to conserving the reproductive potential of the BOFFFs. Conover and Munch (2002) experimentally demonstrated the effects of size-selective harvest and showed that harvest based on a minimum size limit, where all fish over a specific size were taken out of the population, selected for genotypes that had faster growth rates. This ultimately left fish with a slow-growing genotype to reproduce, ultimately reducing the yield (Conover and Munch 2002). Decadal shifts in the growth of red porgy (*Pagrus pagrus*) were also attributed to an extended period of over-exploitation that had selectively removed the fast-growing genotype from the stock, resulting in slower growth and smaller size at age in recent years (Harris and McGovern 1997).

Using an alternative regulatory harvest strategy, such as a harvest-slot limit, could have substantial advantages over a minimum size limit as currently in use. In particular, fish with fast-growing genotypes would pass through the harvest-slot limit (where they are vulnerable to harvest) quickly, and survive to reproduce at a larger size (Conover and Munch 2002); with all the potential reproductive consequences of BOFFFs. Moreover, the overall age structure would recover and older ages would be represented in the population, bringing with it more resiliency to environmental perturbations, as discussed above. Gwinn et al. (2013) recently modelled the benefits that can accrue to both the reproductive sustainability of the stock and the number and size of fish harvested by using a harvest-slot size limit instead of a minimum size limit. They found that a harvest-slot limit consistently produced greater numbers of harvest sized fish while conserving the reproductive biomass of the stock. The harvest-slot size limit also had the added benefit of producing a more natural age-structure of the stock (i.e., not truncated) (Gwinn et al. 2013), which in itself could add to the stock resiliency. Exploring the length range of harvest-slot limits possible would also be instructive since a very narrow harvest-slot limit may not be beneficial or realistic for fishers even though it may help improve the spawning stock biomass.

Harvest-slot limits must also be used judiciously, however, since releasing fish that are larger than the top-end of the harvest slot would be undesirable if those large fish suffered a high degree of release mortality (Berkeley et al. 2004b). Physiologically, release mortality in marine fishes is predominantly associated with barotrauma, where the expansion of the fish's swim bladder on ascent causes physical damage (e.g., stomach protruding from mouth). Fish species with closed swim bladders (i.e., no open connection to their gut tract=physoclistous) are most at risk of barotrauma (e.g., groupers). Greater amberjack also have a closed swim bladder but it is capable of self-venting or releasing gas as the fish ascends to the surface (Murie and Parkyn 2013b). Acute release mortality (i.e., dead on the deck) was observed to be <1% in greater amberjack, even for fish captured at >200 ft (Murie and Parkyn 2013b). Delayed release mortality for greater amberjack also appears to be relatively low compared to other reef fishes and large spawning fish that had been captured at depth, vented, tagged with satellite archival tags, and then released, all survived at least one month (after which the satellite tag was disengaged from the fish) (Murie and Parkyn 2013b). With relatively low release mortality compared to other reef fishes, such as groupers and snappers, over-exploited greater amberjack in the Gulf of Mexico may be an ideal candidate to explore an alternative fishing regulation scenario compared to the current minimum size limit.

## PROJECT GOAL

Our overall goal is to test whether **Bigger, Older, Fatter, and more Fecund Females (BOFFFs)** contribute disproportionately more to the reproductive potential of the spawning stock of greater amberjack in the Gulf of Mexico; and if so, would an alternative management plan based on conserving the reproductive potential of the BOFFFs using a harvest-size slot limit be more efficacious for rebuilding the stock compared to using a minimum size limit? To meet this goal, our project objectives are:

## PROJECT OBJECTIVES

1. Quantify the bigger, older, and more fecund female factor (the BOFFFs) in greater amberjack by modeling the total, potential annual fecundity of Gulf greater amberjack on an age-specific and a size-specific basis. The steps required to do this include:
  - 1a. Determine the duration of the spawning season for female greater amberjack spawning in large aggregations off Louisiana in the Gulf of Mexico.
  - 1b. Estimate age- and size-specific batch fecundity of greater amberjack sampled throughout the spawning season (age- and size-specific estimates).
  - 1c. Estimate the spawning fraction of females throughout the spawning season; use its inverse to estimate the spawning frequency of females (i.e., how many days between spawning batches of eggs) to determine changes during the spawning season.
  - 1d. Estimate the length and size of sexual maturity based on females in both developing and spawning capable phases in relation to ovarian development and growth to re-evaluate previous estimates.
2. Model the “fatter” factor in the BOFFFs using the relationship between the caloric density in their white muscle, liver, and ovaries that can be mobilized as an energy source during the spawning season (used as proxies for better survival of larvae).
3. Incorporate the reproductive parameters into an age/size-structured population model to evaluate the use of a minimum size limit versus a harvest-size-slot (harvest slot) limit aimed at reducing fishing mortality on BOFFFs, and evaluate the effects of these harvest regulations on the number of fish harvested and fisheries yield while maintaining or increasing biomass.

## METHODS

### *Sampling Fish*

Great Amberjack were collected off the coast of Louisiana, USA, during the months of January through the end of June, which encompassed their spawning season (Murie and Parkyn 2008). Fish were caught using typical recreational hook and line fishing. All fish < 500 mm fork length

(FL) were iced and returned to the laboratory for processing because urogenital pores of these small fish do not allow for external sexing in the field (Smith et al. 2014). Fish > 500 mm FL that were identified in the field as definitely male using a non-lethal field sexing technique developed by Smith et al. (2014) were measured for length and released at sea. All amberjack retained were measured for maximum total length (MTL, mm), fork length (FL, mm), weighed (nearest g), and internally sexed based on gonads.

Collection information was also recorded for each sampling event, including date, time, fishing location (latitude and longitude), and depth; a sampling event was defined as fishing at a specific location during the sampling day. Relocating to a different sampling site (i.e., a different oil rig platform) was considered a different sampling event.

### ***Aging Greater Amberjack using Otoliths***

Sagittal otoliths were excised and aged using the protocol developed by Murie and Parkyn (2008); this methodology was consistent with the Gulf States Marine Fisheries Commission (GSMFC) Otolith Working Group Manual (vanderKooy 2009). In summary, one otolith (sagitta) of each pair (usually the left) was embedded in 5-min epoxy resin (Devcon®) in bullet molds, annealed to a fully-frosted slide with Crystalbond® 509 adhesive, and cross-sectioned through the core into 0.5 mm width sections using a Buehler® Isomet 1000 digital sectioning saw. Three sectioning blades fitted with 0.5 mm spacers were used simultaneously in sectioning to ensure consistent section widths and resulted in two sections per otolith that are permanently mounted on slides using Crystalbond. Sections were then covered using Flotexx® to increase optical clarity. Otolith sections were viewed using a stereomicroscope (20-100X) with transmitted light.

Opaque zones were enumerated and the amount of translucent growth on the edge of the otolith was reported as: 1, opaque zone at edge with no translucent margin; 2, translucent growth <1/3 of the previously completed increment; 3, translucent growth >1/3 and <2/3 of the previously complete increment; and 4, translucent growth >2/3 of the completed increment. Fish were then assigned into an age class based on the number of opaque zones and the amount of translucent growth on the edge of their otolith with respect to their collection date and time of opaque zone deposition relative to January 1<sup>st</sup> (vanderKooy 2009). These ages were incorporated into all analyses that were based on age-specific parameters. Aging precision (i.e., repeatability) was assessed by calculating the average percent error (APE) (Beamish and Fournier 1981). An APE <5% is considered adequate for stock assessment purposes for hard to age species, such as greater amberjack (Campana 2001).

### ***Reproductive Sampling and Staging***

Only ovaries were sampled for reproductive staging. Whole ovaries that were excised from each female were damp blotted on a damp paper towel to remove excess moisture and weighed whole. For reproductive staging using histology, two ~1 cm<sup>3</sup> tissue cubes were excised from the medial portion of the largest ovarian lobe, with one tissue cube taken from the periphery (including the ovarian wall) and the second tissue cube was taken from the core of the ovary. Inclusion of the ovarian wall in one of the tissue blocks was critical for staging the sexual maturity of the females (see below). These tissue cubes were fixed for 7 days in 10% neutral buffered seawater formalin

and then transferred to 70% ethanol. Tissue blocks from each ovary were then trimmed and processed in tandem in one cassette using an automated tissue processor at the Louisiana Department of Wildlife and Fisheries Grand Isle Laboratory. Tissues were blocked in paraffin and sectioned using a rotary microtome. Ovarian tissue was sectioned at 6-8  $\mu\text{m}$ , with sections mounted on glass slides, and then stained with haematoxylin and counter-stained with eosin-Y (Humason 1979; Hinton 1990).

Females were reproductively staged for oocyte development using criteria outlined in Brown-Peterson et al. (2011) (Table 1). Staging was done without knowledge of the age, size, or collection date of the females. All fish were scored as to the most advanced oocyte stage present. Fish were then categorized into reproductive phases after Brown-Peterson et al. (2011), including immature and mature (developing, spawning capable, regressing and regenerating) phases (Table 1).

### ***Sexual Maturity***

Size and age of sexual maturity were derived using a logistic regression model based on individual fish determined to be immature or mature as a function of length or age (age class). Maturity of fish was based on three criteria: 1) all females in developing, spawning capable, regressing and regenerating phases (i.e., includes developing fish with CA, V1 and V2 oocytes as mature fish); 2) all females with oocyte stages at or beyond V1 (i.e., all vitellogenic stages considered to be mature, but CA stage considered to be immature); and 3) only oocytes in spawning capable, regressing, and regenerating females (i.e., CA, V1 and V2 fish considered to be immature).

### ***Spawning Season Duration by Age and Size***

To determine the timing and duration of the spawning season for mature greater amberjack in the Gulf, two methods were used: 1) a gross indication of peak spawning using a gonadosomatic index (GSI); and 2) histological analysis using biomarkers.

The GSIs were calculated from the weight of the whole gonad expressed as a percentage of the gonad-free body weight of the female. To correct body weight for potential variability in the presence of gut contents from feeding, stomach contents were also weighed and intestinal contents were stripped and weighed in the laboratory and subtracted from the gonad-free body weight. For all female reproductive analyses hereafter, female weight refers to gonad-free gut-content-free body weight. Mean GSI was plotted by month of capture separately for immature and mature females since the latter group would presumably not show any peak in gonad growth. Peak spawning months and the duration of the spawning season was delineated using the GSI pattern for the mature females.

Histologically, since female amberjack were only sampled during the spawning season, females with oocytes that were only in cortical aveoli (CA) or early vitellogenic (V1 or V2) stages, but without any later stage vitellogenic oocytes (V3), were considered to be in the developing stage (Table 1); females in this stage were developing oocytes for the spawning season but not actively spawning at the time of collection (Brown-Peterson et al. 2011). Once V3 oocytes were observed

in the histological sections, then the female was considered to be in the spawning capable phase (Table 1). The spawning season duration for the greater amberjack stock (“population”) was based on females in the “Actively spawning” subphase of the Spawning Capable reproductive phase (Lowerre-Barbieri et al. 2011). This subphase includes fish that have oocytes in final maturation in stages including germinal vesicle migration (GVM), yolk coalescence and germinal vesicle breakdown (YC/GVBD), hydration (HYDR) and newly collapsed post-ovulatory follicles (POFs) (Brown-Peterson et al. 2011).

### ***Spawning Fraction and Spawning Frequency***

The spawning fraction of mature females was calculated from the number of mature females captured that were actively spawning (e.g., Actively spawning subphase) divided by the total number of all mature females (Fitzhugh et al. 2009); samples had to be pooled by month due to sample size. The inverse of the spawning fraction was used to calculate the average interval (in days) between spawning of batches of eggs. Spawning fractions were plotted as a function of month to determine if the proportion of females actively spawned changed over the spawning season.

### ***Batch Fecundity Estimates by Size and Age***

Batch fecundity was estimated using a modified hydrated oocyte method on a gravimetric basis (Hunter and Macewicz 1985; Hunter et al. 1985; Fitzhugh et al. 2009). For Greater Amberjack, the incidence of observing hydrated oocytes in spawning capable females was reported by Harris et al. (2007) to be less than 2% and therefore oocytes in the germinal vesicle migratory (GVM) stage or yolk coalescence/germinal vesicle breakdown (YC/GVBD) stage (collectively referred to as GVM+) were used instead of hydrated oocytes, following Harris et al. (2007). In summary, the number of GVM+ oocytes in a precisely weighed subsample of ovarian tissue was extrapolated to the whole ovary weight for each female. To do this, ovarian tissue of ~5 g each were excised from the medial portion of each lobe of the ovary following the sampling for the reproductive histology subsamples. These fecundity subsamples were fixed in 10% neutral buffered seawater formalin. Harris et al. (2007) have previously demonstrated that the density of late-stage oocytes is independent on sampling location in the ovaries.

For fecundity analysis, an approximately 0.075-0.10 g of fixed ovary was removed from the formalin and blotted on a dry Kimwipe to remove residual fluid. This methodology followed the South Carolina Department of Natural Resources protocol used for greater amberjack fecundity analysis in the South Atlantic (pers. comm., D. Wyanski). This blotted piece of ovary was then weighed precisely (0.0001 g) and the entire sample placed in a labelled vial with a solution of 33% glycerin:water. This solution helped to separate the oocytes from the ovarian membranes and extraneous tissue (Collins et al. 1998). After 2-3 days in the glycerin solution the oocytes were cleared enough that the GVM+ oocytes could be differentiated from V3 oocytes. The entire sample was then washed into a plankton-counting S-chamber for enumeration using a stereomicroscope at 25X magnification. All oocytes in GVM, yolk coalescence, germinal vesicle breakdown, or hydration stages were enumerated.

Oocytes in GVM+ stages were summed for each fecundity count and divided by the subsample weight to determine the number of oocytes per gram ovarian tissue. Two independent subsamples and counts were done for each female and averaged to estimate a mean number of oocytes per g-ovary for each female. The estimated average oocytes/g-ovary was then multiplied by the total ovary weight of the female to estimate the batch fecundity, which is the total number of GVM+oocytes per female for one batch of eggs. Females identified as having recent post-ovulatory follicles (approx. <24 hrs old), which indicate that some of the oocytes have most likely already been spawned during the current spawning day, were not used in the batch fecundity estimates. Batch fecundity was modelled as a function of female weight, length, and age.

### ***Female Condition in Relation to Reproductive Stage***

In addition, female condition was assessed using caloric density of ovarian, muscle and liver tissues. A subsample of up to 20 g was excised from the ovary, liver, and anterio-dorsal white muscle of each female and frozen until processed. Ovarian tissue was sampled concurrent with tissues for histological and fecundity analyses. All thawed tissues were ground using a food blender, placed in labelled whirl paks, and weighed precisely (0.0001 g). Samples were then freeze-dried to constant weight and the freeze-dried weight was used to determine % moisture in the tissues. Caloric density of tissues (kcal per gram dry weight) was determined using an isoperibol calorimeter using standard protocols (Parr Instruments). Duplicates were run on a subset of ovarian, muscle and liver tissues to determine precision of caloric determinations. Caloric density (kcal/g) of these tissues was modelled as a function of female size during the spawning season.

### ***Minimum-size Limit versus Slot-size Limit***

An age/size-structured population model was used to evaluate the use of a minimum size limit versus a harvest-size-slot (harvest slot) limit aimed at reducing fishing mortality on BOFFFs, and to evaluate the effects of these harvest regulations on the number of fish harvested and fisheries yield. The basic age-/size-structured population model that will be used to compare fisheries yields based on implementing a harvest-slot limit versus a minimum size limit will be based on Gwinn et al. (2013) with modification from Coggins et al. (2007). Gwinn et al.'s (2013) model is structured to determine the "optimal" length-based fishery regulations that would satisfy management for both the fishery (i.e., harvest rates) while sustaining the reproductive potential of the population and minimizing age (and size) truncation. The model accounts for sources of mortality, including natural mortality, mortality due to harvest directly, and release (discard) mortality. It also incorporates length-based vulnerabilities to the fishery and density-dependent compensation in recruitment (Gwinn et al. 2013). One benefit of using a harvest-slot limit is that the fastest growing fish pass through the harvest slot, where they are vulnerable to harvest and removal from the population, relatively quickly compared to slower growing individuals (Conover and Munch 2002).

The flexibility of the model to use a range of minimum size limits and range of harvest-slot size limits to find an optimal compromise between immediate fisheries needs (i.e., harvest) and conservation of the spawning stock for rebuilding could provide unexpected alternatives to

consider in the management of greater amberjack. This may be particularly instructive for greater amberjack in the Gulf of Mexico since the minimum size limit is currently 34 inches fork length, relatively high for a minimum size limit for a “reef” fish.

## RESULTS AND DISCUSSION

### *Fish Sampling*

A total of 1,024 amberjack were captured from January through June in 2016 and 2017. Of these, 569 were females and 383 were males returned to the lab for processing, with 72 fish identified as males (producing milt) measured and released at-sea. Female greater amberjack captured in January to June, 2016 and 2017, ranged in size from 300-350 mm fork length (FL) to 1350-1400 mm FL (Figure 1A). Male greater amberjack captured in January to June, 2016 and 2017, ranged in size from 300-350 mm fork length (FL) to 1300-1350 mm FL (Figure 1B).

Age frequencies of females ranged from 1 to 9 years, with the majority of fish in age classes 2-5 (Figure 2A). Male amberjack ranged in age from 1 to 11 years of age, with the majority of males between 2 and 4 years of age (Figure 2B).

Female greater amberjack ranged from 640 to 36,585 g in total body weight corrected for the weight of any stomach or intestinal contents. The power function of weight and length was log<sub>10</sub>-transformed to correct for heteroscedasticity and non-normality and an Analysis of Covariance (ANCOVA) was used to test for differences between years and between males and females. The logweight (g) as a function of logFL (mm) relationship for females collected in 2016 and 2017 was not significantly different (ANCOVA: slopes  $P=0.085$ ; intercepts  $P=0.058$ ) and years were therefore pooled.

Male amberjack ranged in weight from 840 to 32,340 g. Total body weight was not corrected for stomach or intestine contents for males because they were processed in the lab for length, weight, and age (otoliths) only when they could not be confirmed as male in the field. Logweight as a function of logFL was not significantly different between years (ANCOVA: slopes  $P=0.100$ ; intercepts  $P=0.055$ ), and years were therefore pooled.

The relationship between weight as a function of length for female and male amberjack was not significantly different (ANCOVA: slopes  $P=0.862$ ; intercepts  $P=0.861$ ), and sexes were therefore pooled (Figure 3).

### *Sexual Maturity*

Based on the criterion of females in a developing phase or beyond being sexually mature, the smallest mature female was 710 mm FL and the largest was 1373 mm FL (Figure 4). The largest immature female was 994 mm FL, with immature females ranging to 340 mm FL.

Size-at-maturity for females designated as mature based on being in the developing phase and beyond (cortical alveolar oocytes, CA, and beyond) (Figure 5A) was slightly less at 50%



maturity (~827 mm FL) than for females designated as mature based on having early stage vitellogenic oocytes (V1 and V2, and beyond) but not CA oocytes (~861 mm FL) (Figure 5B). These latter females were in turn slightly less than for females designated as mature based on reaching spawning capable (V3 and beyond) (~873 mm FL) (Figure 5C). The largest difference was the inclusion of females that had oocytes in the cortical alveolar (CA) stage based on being physiologically mature.

### ***Spawning Season***

Females that were physiologically mature and developing cortical alveolar oocytes were present throughout the spawning season, although in much higher percentages in January and February (Figure 6). Early vitellogenesis (V1 and V2) was greatest in January and February as well, with spawning capable females with late vitellogenic oocytes (V3) appearing as early as January. However, females were not actively spawning until March, which is when females with oocytes undergoing germinal vesicle migration (GVM) or yolk coalescence and germinal vesicle breakdown (YC/GVBD) were first observed. There were very few hydrated females observed in the study (n=4), which is very similar to Harris et al. (2007) in their study of spawning South Atlantic Greater Amberjack. Atresia associated with post-spawning events was observed as early as March and the majority of mature females had atretic oocytes by June (Figure 6). Based on histological study of individual oocyte types in the ovaries of mature females, the peak spawning season was in March, April and May.

The peak spawning season was clarified by using reproductive phases, which designates mature females as developing (not spawning capable at the time of collection), spawning capable (will spawn in the current spawning season), and post-spawning females (regressing and regenerating) (Figure 7). A large percentage of females (~80-90%), well over 50%, are in the spawning capable phase by March and continue into May.

The gonadosomatic index (GSI) for mature female greater amberjack during January to June, 2016 and 2017, was significantly different among months (ANOVA:  $P < 0.0001$ ). GSIs in March, April and May were significantly greater than GSIs in January, February and June (SNK:  $P < 0.05$ ) (Figure 8), indicating peak spawning during March through May. This was consistent with observations on the most advanced oocyte stage of mature female greater amberjack that also indicated spawning capable fish during March, April, and May (Figure 7). In contrast, immature females had GSI values  $< 0.5\%$  of their body weight throughout the spawning season, and as predicted there was very little variation in their GSI over that period (Figure 8).

The increase in ovary weight as a function of female size (Figure 9) visually confirmed both the size at sexually maturity and the peak spawning season. Ovary weight did not increase significantly until females reached ~800-850 mm FL, and increased significantly in March, April and May.

The duration of the spawning season was estimated as ~76-77 days based on the observation of females in the actively spawning subphase (GVM, YC/GVBD, Hydrated, newly collapsed POFs) (Table 2). When all spawning capable females were included (i.e., those with V3 oocytes as well), then the duration of the spawning season increased (94-134 days), especially in 2017 with

more intensive sampling. Harris et al. (2007) similarly estimated the spawning season to be ~73 days off of South Florida (27 February – 10 May), basing it on the criteria of females having to be in the actively spawning subphase (in final oocyte maturation). Off Louisiana, females in final oocyte maturation were not caught until mid-March in either 2016 or 2017, although they were caught into late May and early June (Table 2).

### ***Spawning Fraction and Spawning Frequency***

Females were only in the actively spawning subphase during March through June, and so the spawning fraction in January and February was zero (Figure 10). This would need to be taken into account when estimating the reproductive potential of greater amberjack because although they have larger ovaries in January and February (giving the appearance of spawning), their oocytes were in late vitellogenesis but not in final maturation indicating imminent spawning (Figure 6). The spawning fraction was fairly consistent over the peak spawning months of March, April, and May (0.20-0.25) and then markedly decreased to 0.083 in June, but only 12 mature females were captured in June. However, the decrease in spawning fraction is in concert with the decrease in spawning activity observed in the histological and GSI analyses (Figures 6, 7, and 8). Harris et al. (2007) observed a similar spawning fraction for greater amberjack in the South Atlantic, reporting an average of 0.227.

Based on the spawning fraction, the interval between spawning batches of eggs was consistent among the months of March through May, and then increased markedly as the spawning fraction decreased in June (Figure 10). On average, greater amberjack were capable of spawning every 4.5 days through the months of March to May, with the interval increasing to ~12 days between spawning events in June. Similarly, Harris et al.'s (2007) average spawning fraction corresponded to a spawning interval of ~5 days.

### ***Batch Fecundity Estimates by Size and Age***

The relationship between batch fecundity and female fork length was positive and significant ( $r^2 = 0.58$ ,  $P < 0.05$ ) (Figure 11). Overlaying the batch fecundity regression reported in Harris et al. (2007) showed that greater amberjack in the South Atlantic have a greater batch fecundity in relation to their size compared to greater amberjack off Louisiana (Figure 12). Similarly, batch fecundity as a function of female weight was significant, linear, and positive ( $r^2 = 0.72$ ,  $P < 0.05$ ) (Figure 13). Batch fecundity estimates as a function of fish age was more variable but was still significantly positive ( $r^2 = 0.33$ ) (Figure 14). Sample size was low for younger fish because of sexual maturity and low at older ages because the majority of greater amberjack were  $\leq 6$  years of age (Figure 2A).

### ***Female Condition in Relation to Reproductive Stage***

Fish typically use lipids as a source of energy, storing it in their large white muscle tissues and mobilizing it for use in oocyte development and energy acquisition during the spawning season.

Overall, caloric density of muscle tissue only showed a slight increase in relation to female size in all months collected, except March (Figure 15). Muscle energy density was greatest in February, prior to peak spawning. In March, at the beginning of peak spawning, muscle energy density was still relatively high but showed no relationship with fish size. Muscle energy density was lowest in June during the post-spawning period.

In addition to white muscle tissue, fish store energy dense lipids in their liver tissue and mobilize it as a source of energy for oocyte development for spawning and other activities (i.e., migration). Females in February and to a lesser extent January, prior to the start of peak spawning, had higher levels of energy reserves in their liver tissue than other months and the liver energy density increased with female size (Figure 16). In March, at the start of peak spawning, liver energy density was lower than February for most females but showed a slightly negative downward trend with female size. Liver energy densities in the peak spawning months of April and May were low and comparable to that of June in the post-spawning period. This indicated that females were most likely transferring energy from their lipid-rich livers to their ovaries starting in March.

Caloric density of ovarian tissue showed an increase in relation to female size during the months of March-May (Figure 17). In addition, females that had increased ovary weight in March, April and May (females approx. > 860 mm FL) (Figure 9) had ovaries with higher caloric density than similar-sized females in January, February and June. This is presumably due to the presence of lipids (i.e., high energy density) in the ovaries (oocytes) of females ready to spawn. This was supported by the decrease in liver energy density during the same period (Figure 16). There appears to be a size threshold for females (~800 mm FL) in having ovaries with high energy density during the peak spawning months and this may be directly related to their ability to spawn (or not, if energy density is too low). In contrast, some large females (>1000 mm FL) had low ovarian energy density during the peak spawning months of March-May. These females were all mature based on prior spawning indicators, but they were not spawning capable when collected and some were not predicted to spawn in the current spawning season based on histological analysis (i.e., potential skipping).

### ***Minimum-size Limit versus Slot-size Limit***

We explored the potential of a model derived from Gwinn et al. (2013) to examine the effect of the minimum size limits and Harvest Slot width on the possible proportion of fish available for harvest and the proportion of large-sized fish available. To test the potential for this method of management, we have initially examined three minimum length limits: 28" (711 mm), 34" (863 mm), and 36" (914.4 mm). Under current regulations, FL = 34" (863 mm) and 36" (914 mm) are regulatory length minimums for recreational and commercial fisheries respectively and fish above these length minimums are open to harvest.

Regardless of minimum size, as the slot size widens, the potential number of fish harvested increases rapidly (Figure 18 A-C). A greater minimum size does provide some reduction in harvest and a bit more protection to large reproductive fish. However, a smaller minimum size coupled with a narrow slot size, limiting retention, may lessen the probability of retention of

large reproductive fish, increasing their prevalence in the reproductive pool relative to a minimum size limit only scenario. Therefore, a narrow slot limits the potential proportion of the population available for harvest and increases the number of large fish that are available to spawn. This observation supports the previous findings of Gwinn et al. (2013), who observed a similar pattern in several species. In essence, it appears that under the scenario of high fishing mortality experienced presently by Gulf of Mexico Greater Amberjack, the implementation of a narrow retention slot would increase the proportion of larger fish in the population and limit the harvest overall. Again, this is largely a result of minimum size regulations allowing the retention of any fish including the largest, most fecund fish from the population, while a slot limit greatly affects the number of retained fish. A slot limit may be a means to reduce or end retention of BOFFFs, because the majority of fish over 1 m are females (Smith et al. 2014). Given the low hooking mortality rate of Greater Amberjack in vertical-line fisheries (SEDAR 2014), this may be a strategy that can be implemented for such fisheries. It would be more problematic to implement for spearfishing, which accounts for some of the GAJ landings, as well as long-line fisheries which have a higher hooking mortality rate. This proof of concept in the use of a harvest slot for Greater Amberjack could be explored more fully with a stock assessment approach to simulate alternative slot sizes in relation to rebuilding spawning stock biomass.

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Table 1. Reproductive staging criteria used for greater amberjack (Brown-Peterson et al. 2011, with minor modification).

| Phase            | Interpretation   | Macroscopic Features  | Histological Features  |
|------------------|--|---|--|
| Immature         | Never spawned  | Small ovaries, often clear, blood vessels indistinct  | Only oogonia and PG oocytes present; no atresia or muscle bundles; thin ovarian wall and little space between oocytes  |
| Developing       | Ovaries beginning to develop but not ready to spawn                                  | Enlarging ovaries, blood vessels becoming more distinct                                     | PG, CA, V1 and V2 oocytes present; no evidence of POFs or V3 oocytes; some atresia may be present<br><i>PG and CA oocytes only</i>   |
| Spawning Capable | Developmentally and physiologically able to spawn in this cycle                      | Large ovaries, blood vessels prominent; individual oocytes visible macroscopically          | V3 oocytes present or POFs present; atresia of vitellogenic and/or hydrated oocytes may be present; early stages of oocyte maturation may be present<br><i>Oocytes undergoing late GVM, GVBD, HYDR, or ovulation</i> |
| Regressing       | Cessation of spawning  | Flaccid ovaries, blood vessels prominent  | Atresia (any stage) and POFs present; some CA and/or V1 and V2 oocytes present   |
| Regenerating     | Sexually mature, reproductively inactive (resting prior to the next spawning season) | Small ovaries (but still much larger than immature fish), blood vessels reduced but present | Only oogonia and PG oocytes present; muscle bundles, enlarged blood vessels, thick ovarian wall and/or late atresia or old, degenerating POFs may be present   |



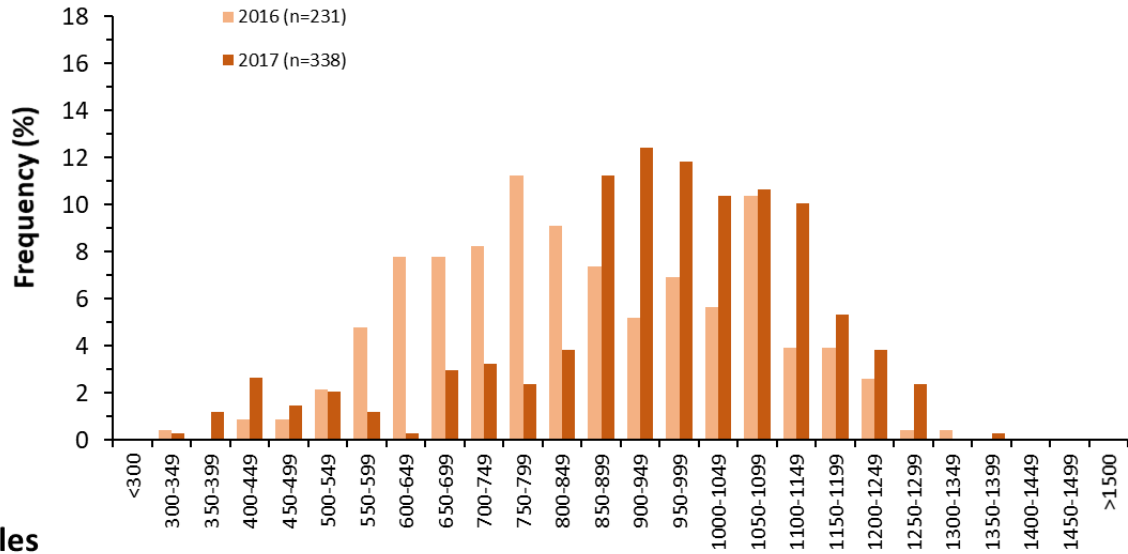
Table 2. Active spawning season of female greater amberjack off of Louisiana during 2016 and 2017 based on females in the actively spawning subphase (oocytes in GVM, YC/GVBD, HYDR, or new POFs) or females in the spawning capable phase, which also includes females with late vitellogenic oocytes (V3).

| <b>Phase/Subphase</b>  | <b>Year</b> | <b>n</b> | <b>Earliest Date</b> | <b>Latest Date</b> | <b>Duration (Days)</b> |
|--|-------------|----------|----------------------|--------------------|------------------------|
| Actively Spawning Subphase (does not include fish with V3 oocytes)                                 | 2016        | 15       | 16 March             | 1 June             | 77                     |
|  | 2017        | 46       | 16 March             | 31 May             | 76                     |
|  | Pooled      | 61       | 16 March             | 1 June             | 77                     |
| Spawning Capable Phase (includes fish in the Actively Spawning Subphase and those with V3 oocytes) | 2016        | 72       | 28 Feb               | 1 June             | 94                     |
|  | 2017        | 173      | 31 Jan               | 14 June            | 134                    |
|  | Pooled      | 245      | 31 Jan               | 14 June            | 134                    |

Table 3. Model parameters for simulation of slot-size effects on proportion of large Greater Amberjack, and effects of proportion of harvest. Source of parameters is SEDAR (2016).

| Parameter                              | Recreational Slot                 | Commercial Slot                   | Sub-mature Slot  |
|--|-----------------------------------|-----------------------------------|--|
| $L_{\infty\max}$                       | 1621.92                           | 1621.92                           | 1621.92  |
| $L_{\infty\min}$                       | 1081.28                           | 1081.28                           | 1081.281   |
| $L_{\infty\text{mean}}$                | 1351.6                            | 1351.6                            | 1351.6   |
| k                                      | 0.22                              | 0.22                              | 0.22   |
| i                                      | 20L                               | 20L                               | 20L  |
| crmort                                 | 0.3                               | 0.3                               | 0.3  |
| cvgroups                               | 0.1                               | 0.1                               | 0.1  |
| M                                      | 0.28                              | 0.28                              | 0.28   |
| Max age                                | 12                                | 12                                | 12   |
| Max size                               | 1600                              | 1600                              | 1600   |
| Length at maturity                     | 825                               | 825                               | 825  |
| lmatfrac                               | 1.484                             | 1.484                             | 1.484  |
| Minimum legal size<br>(bottom of slot) | 34" (864 mm)<br>recreational size | 36" (914.4 mm)<br>commercial size | 28" (711 mm) sub-legal<br>under<br>current regulations |
| Upper size of Slot                     | 1200                              | 1200                              | 1100   |
| S                                      | 0.75574                           | 0.75574                           | 0.75574  |
| $t_0$                                  | -1.83                             | -1.83                             | -1.83  |
| $r_0$                                  | 1e+06                             | 1e+06                             | 1e+06  |
| $W_a$                                  | 3.6e-05                           | 3.6e-05                           | 1e+06  |
| $w_b$                                  | 2.91                              | 2.91                              | 2.912  |

### A) Females



### B) Males

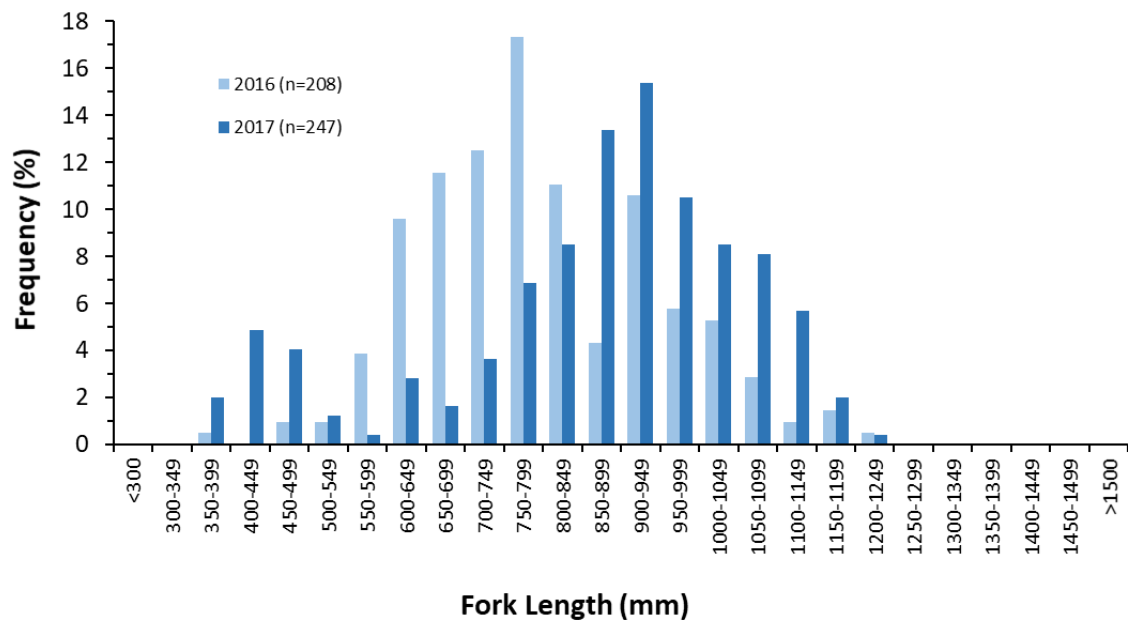
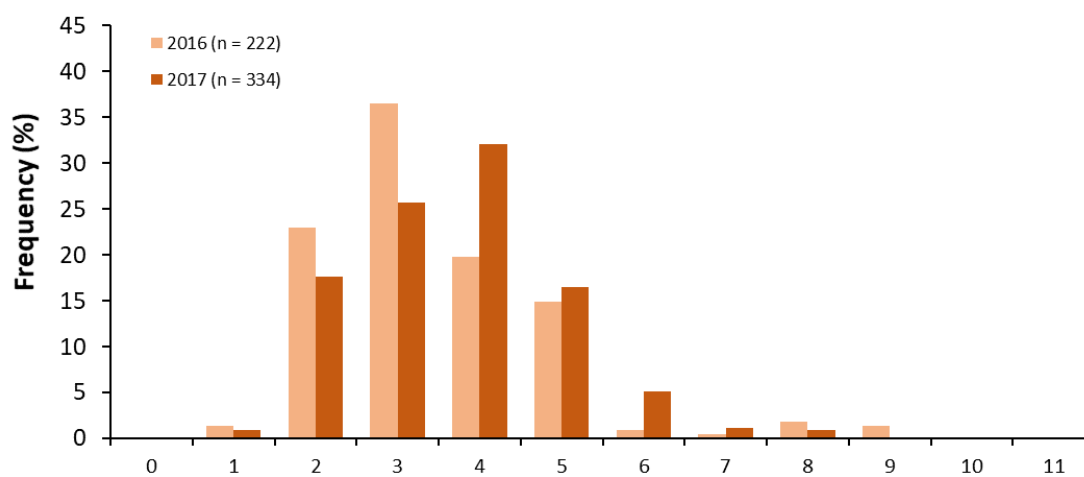


Figure 1. Length frequencies of A) female and B) male greater amberjack collected in Louisiana during January through June, 2016 and 2017.

### A) Females



### B) Males

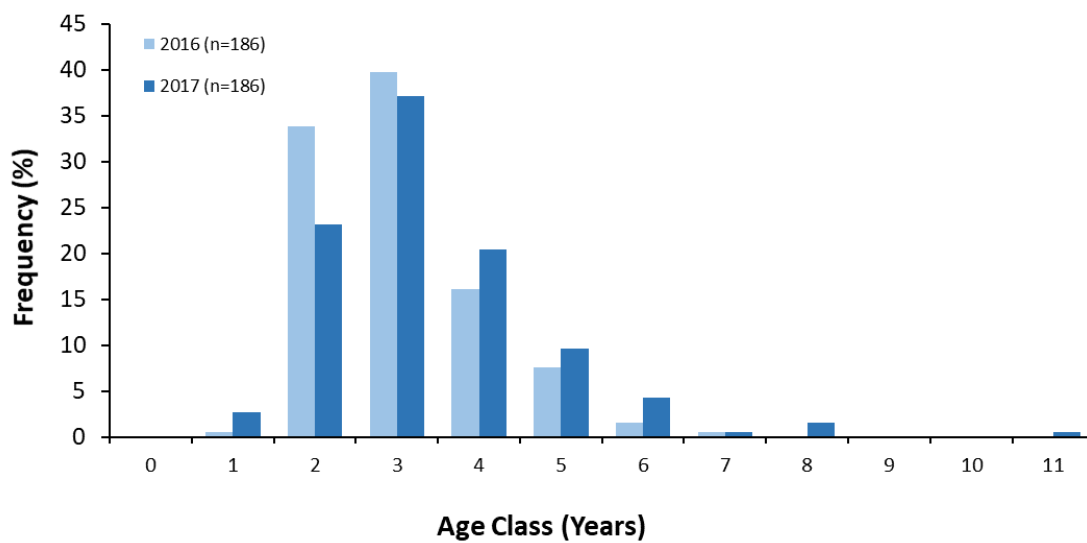
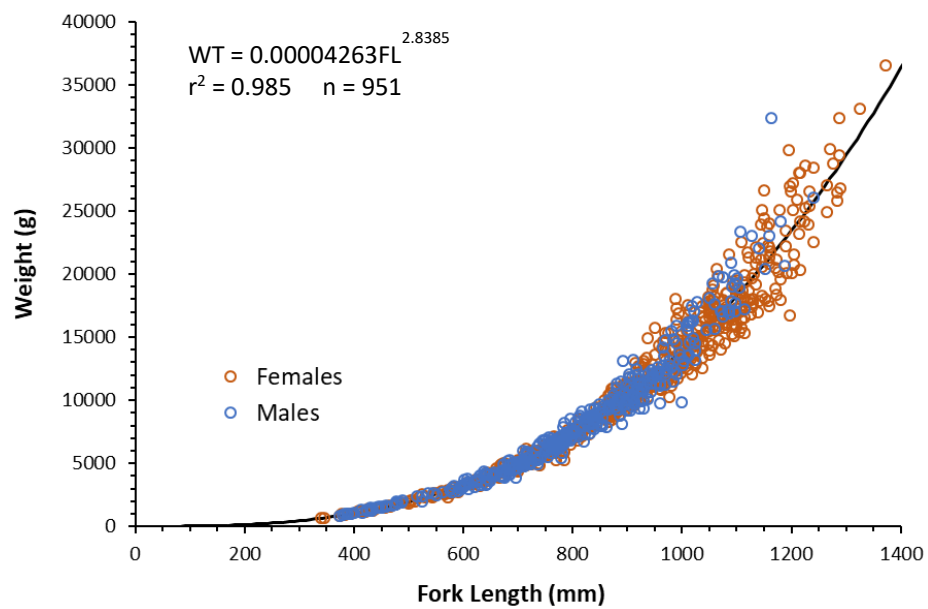


Figure 2. Age frequencies of a) female and B) male greater amberjack collected in Louisiana during January through June, 2016 and 2017.



*Figure 3. Weight as a function of fork length for A) female, B) male, and C) female and male greater amberjack collected in Louisiana from January through June, 2016 and 2017.*

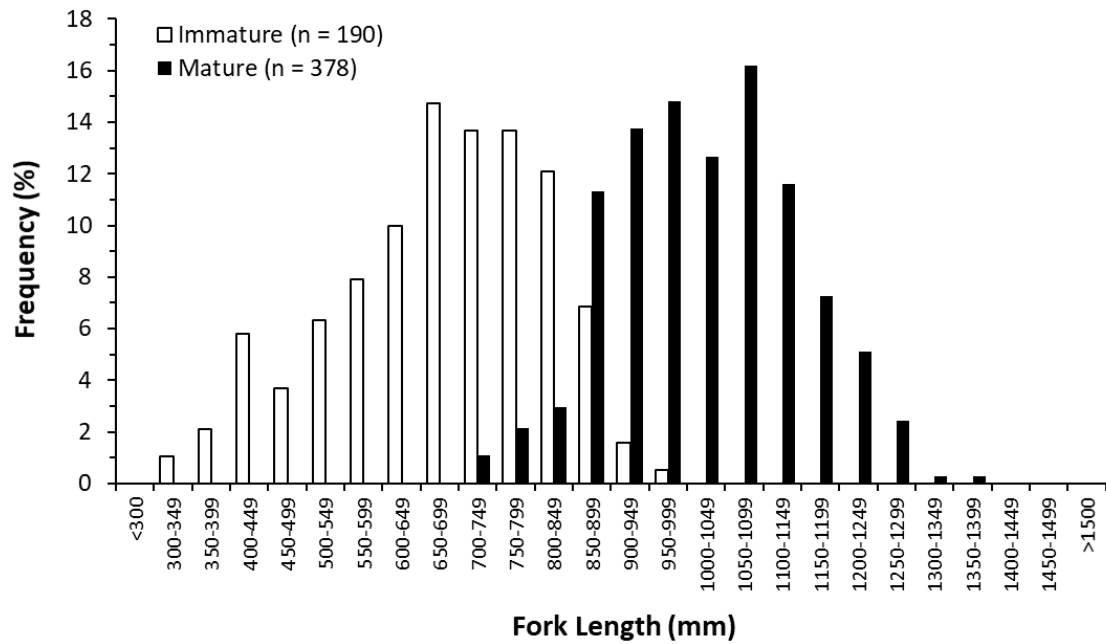


Figure 4. Length frequencies of female Greater Amberjack assigned as immature and mature.

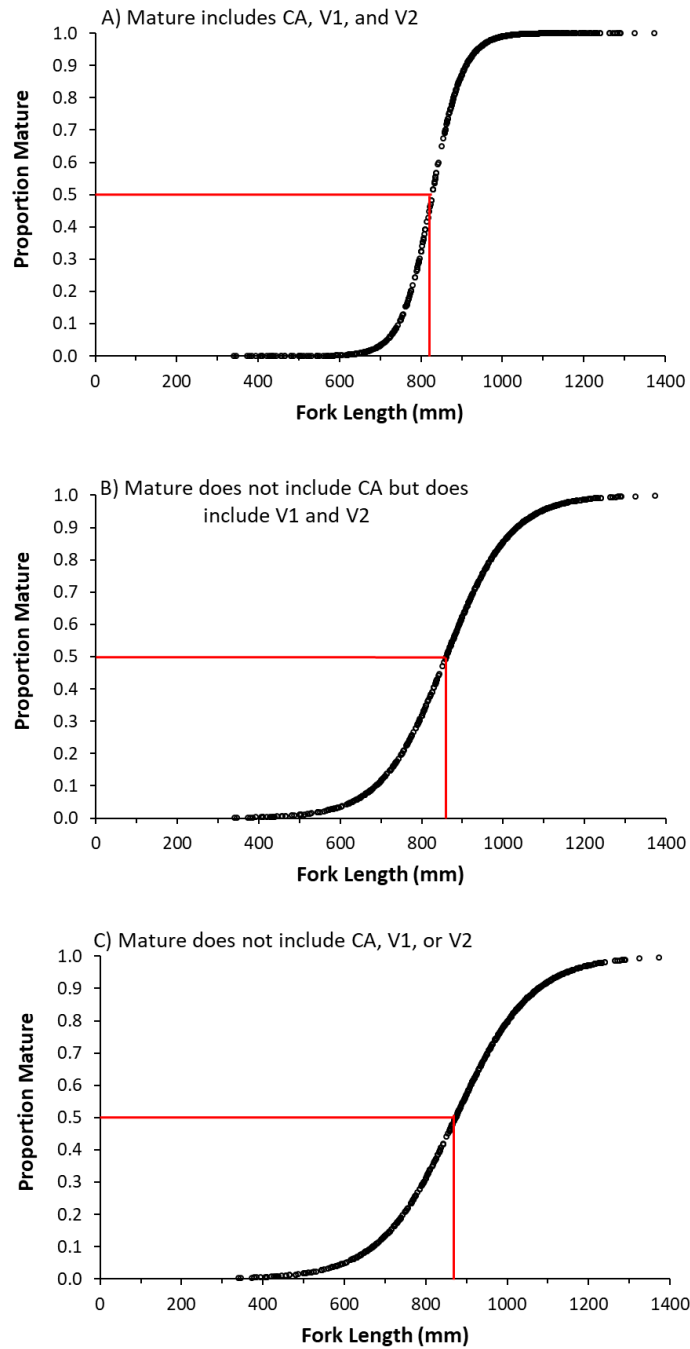


Figure 5. A) Estimates of sexual maturity of greater amberjack as a function of fish FL for females with oocytes in the developing phase or beyond; B) in vitellogenesis and beyond; and C) in spawning capable phase and beyond when sampled in January to June 2016 and 2017. Horizontal reference line is at 50% maturity, with the red vertical arrow indicating intersection with FL.

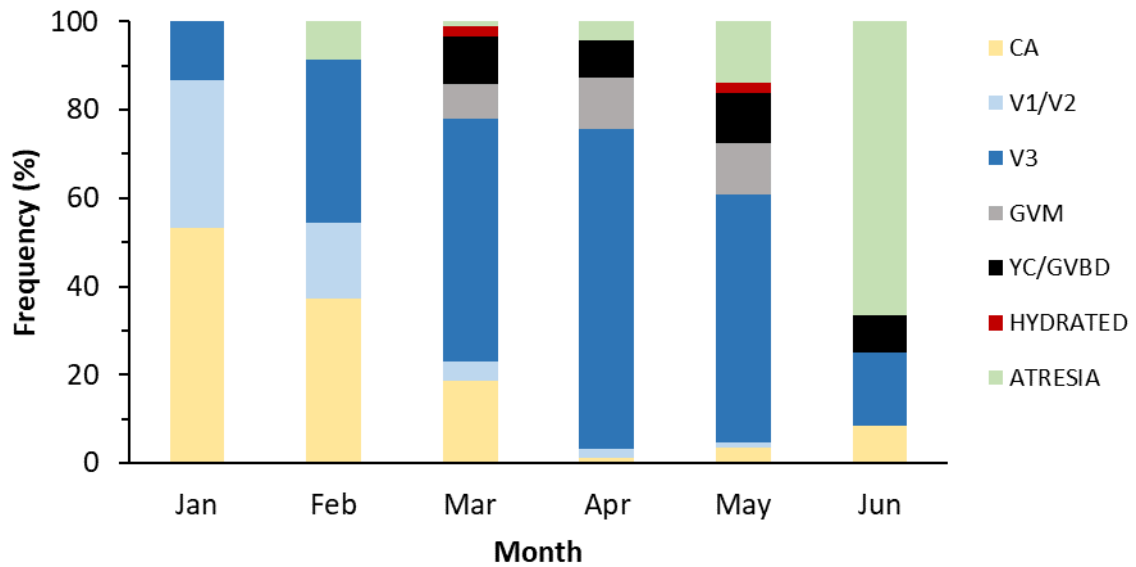
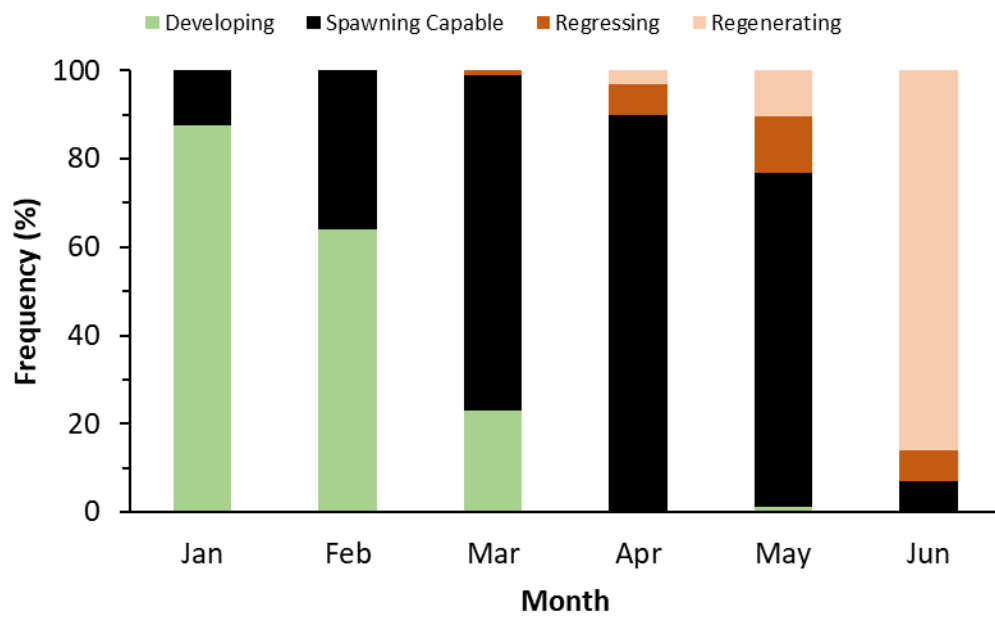


Figure 6. Most advanced oocyte stage observed in histological sections from mature female greater amberjack by month of capture in Louisiana, with CA=cortical alveolar, V1/V2=stage 1 and 2 vitellogenic oocytes, V3=stage 3 vitellogenic oocytes, GVM=germinal vesicle migration, YC/GVBD= yolk coalescence with germinal vesicle breakdown, HYDRATED=hydrated, and ATRESIA = primarily alpha and beta atresia.





*Figure 7. Reproductive phases observed in histological sections from mature female greater amberjack by month of capture off Louisiana.*

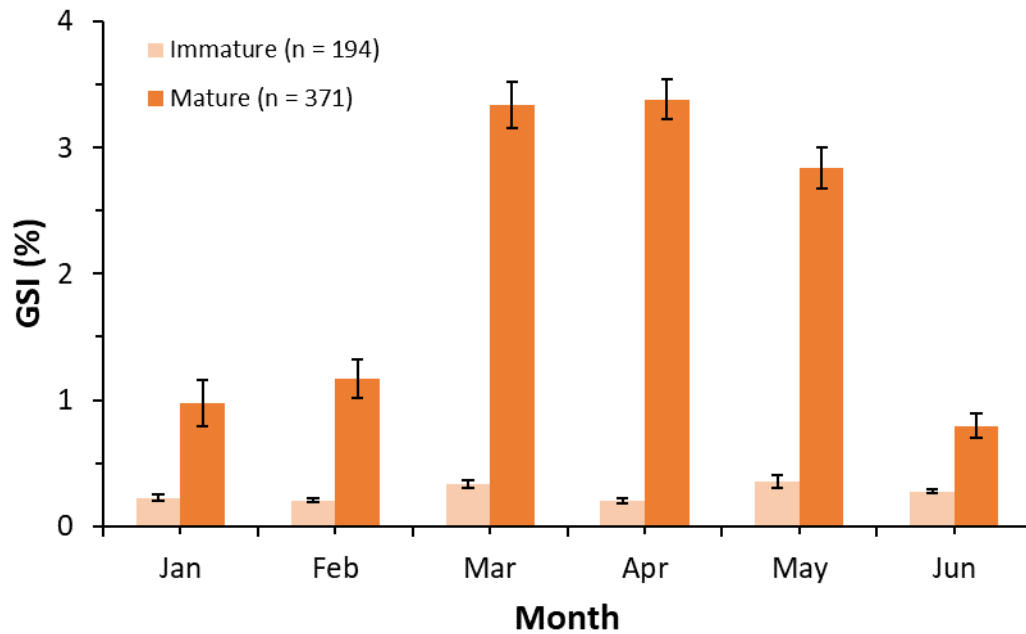
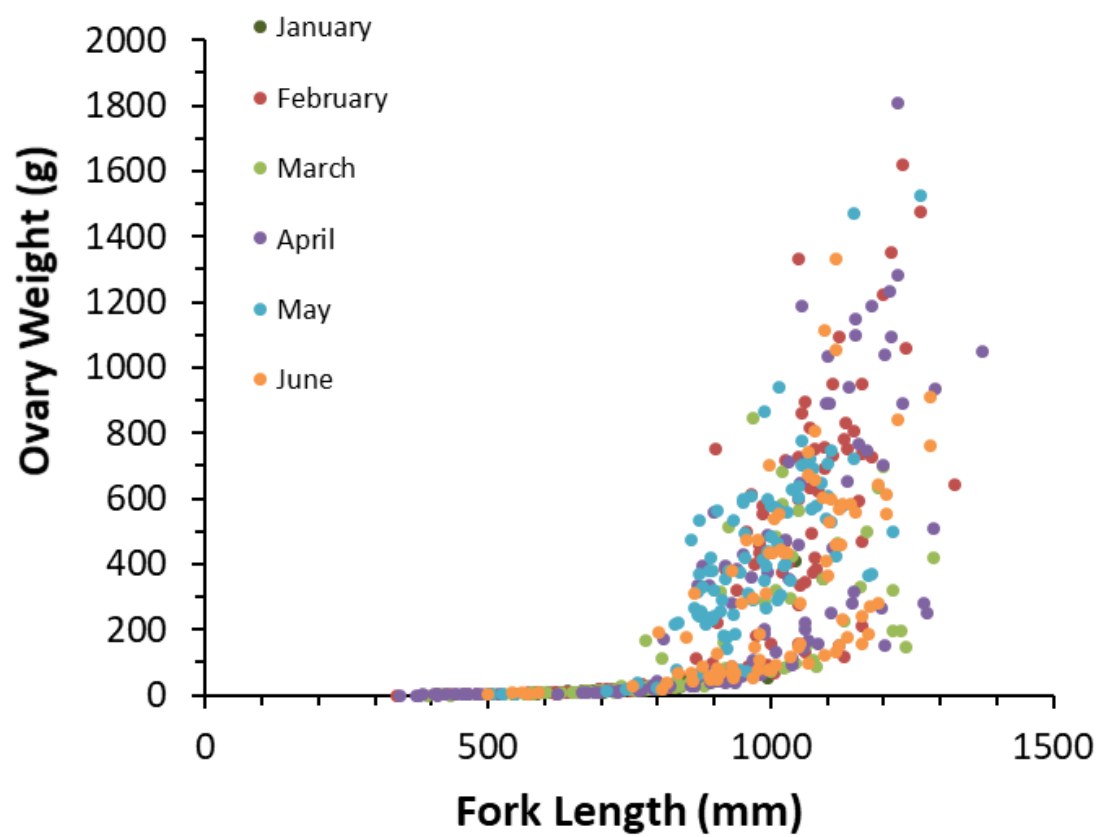


Figure 8. Mean GSI (gonadosomatic index) for female greater amberjack collected in Louisiana during the 2016 and 2017 spawning seasons. Vertical bars are  $\pm 1$  SE.



*Figure 9. Ovary weight as a function of fork length for female amberjack collected in January through June, 2016 and 2017.*

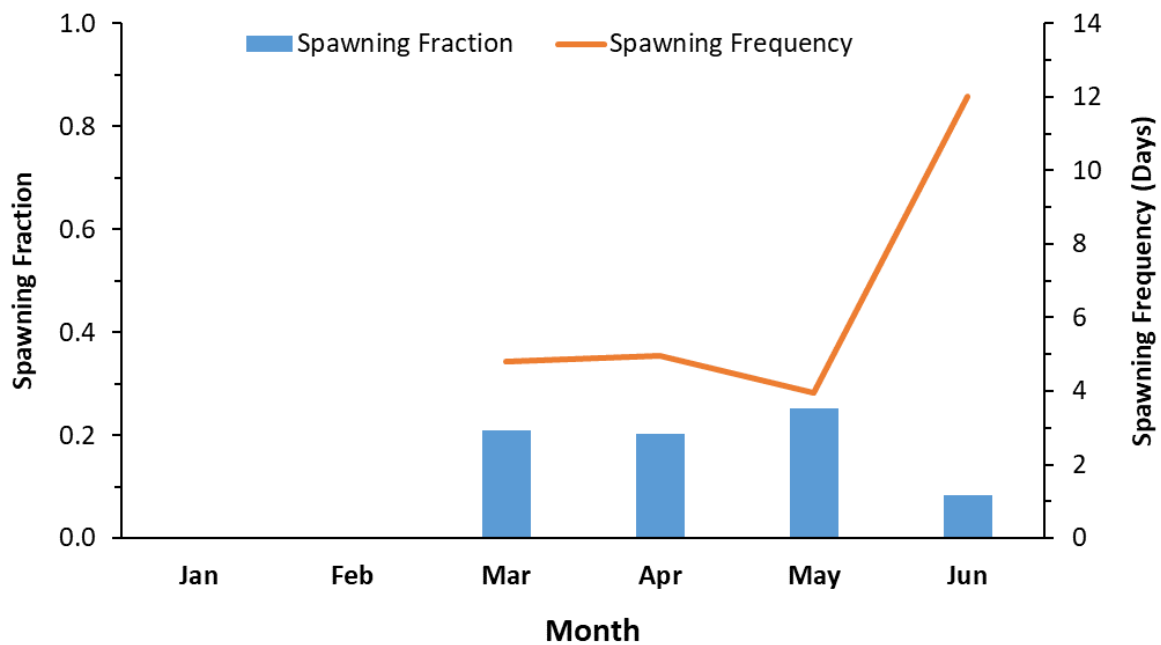


Figure 10. Spawning fraction for greater amberjack in coastal waters of Louisiana during the 2016 and 2017 spawning season. Spawning frequency was calculated for the corresponding months based on the assumption that females retain spawning biomarkers for 24 hr (1 day).

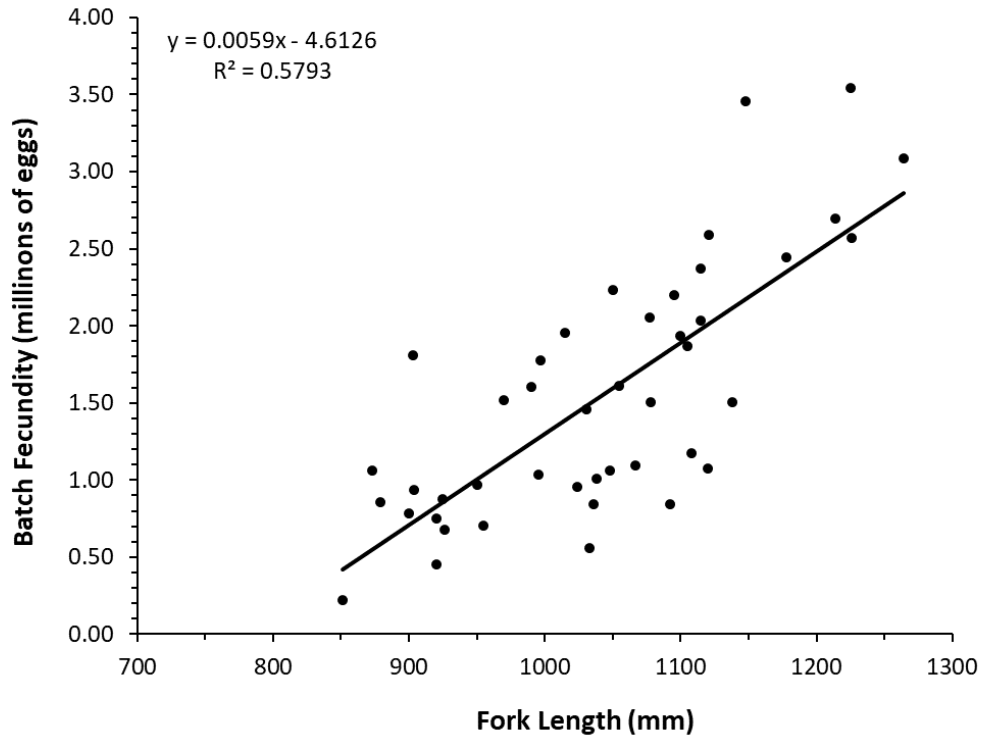
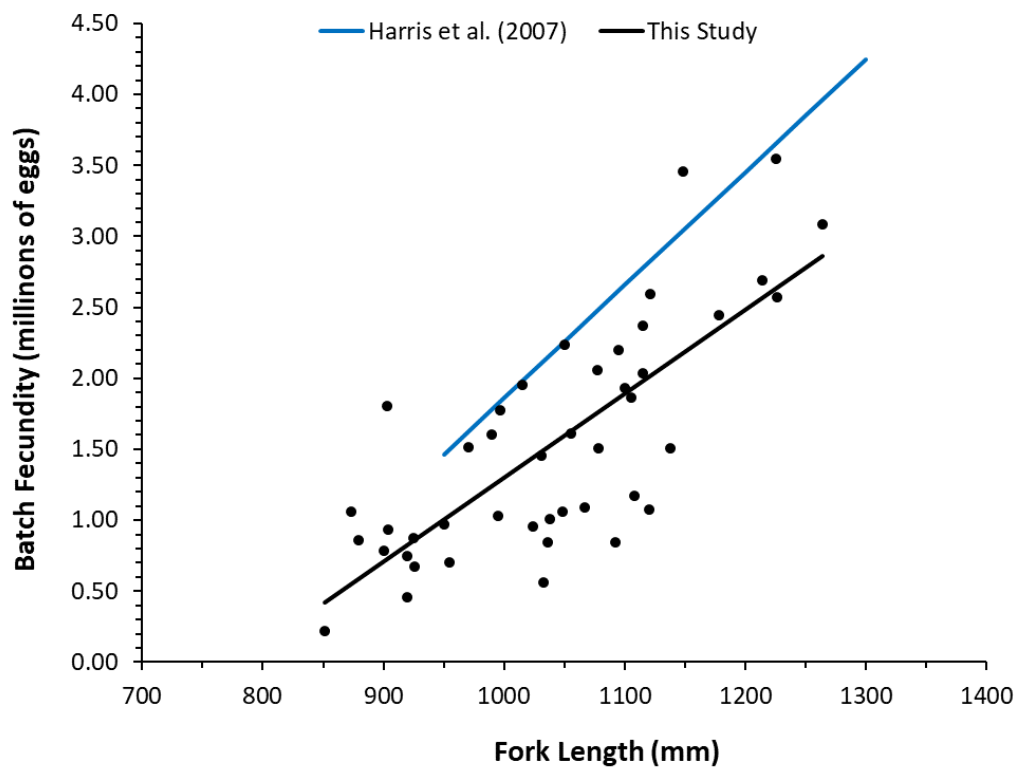


Figure 11. Batch fecundity as a function of female fork length for greater amberjack sampled off of Louisiana in 2016 and 2017 during the January to June spawning season ( $n=44$ ).



*Figure 12. Estimated batch fecundity as a function of fork length (mm) for greater amberjack (n=44) with oocytes in final oocyte maturation (GVM and beyond).*

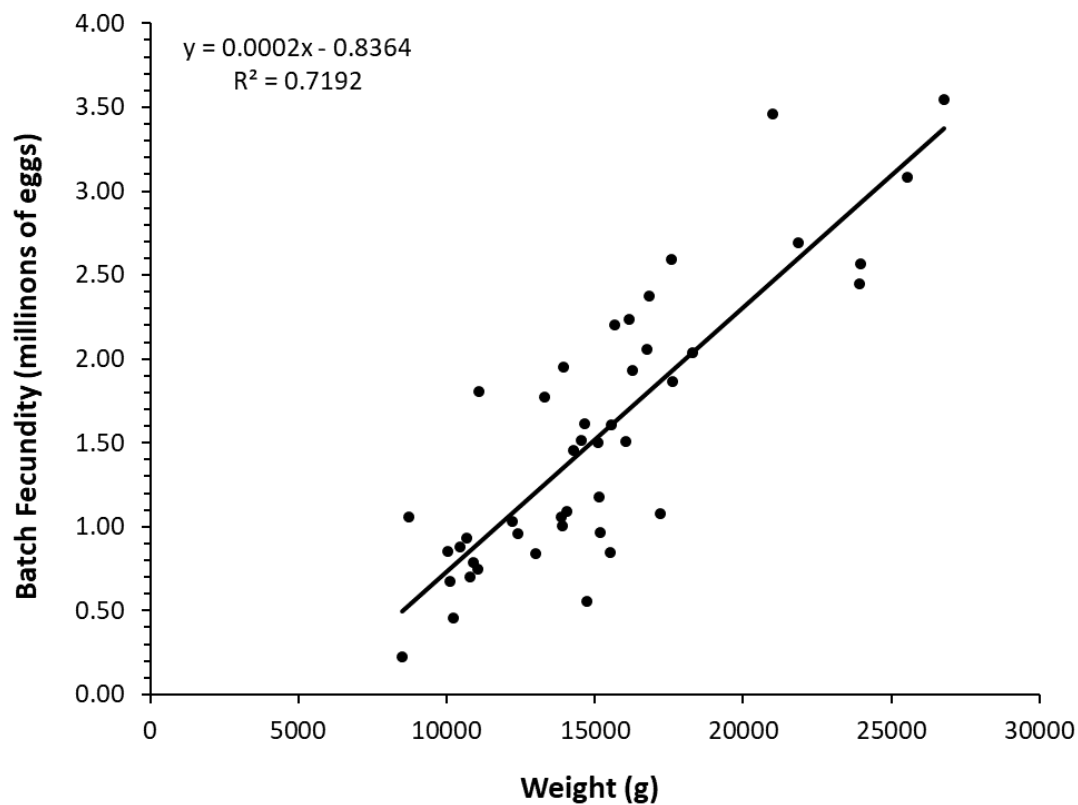


Figure 13. Estimated batch fecundity as a function of weight (g) for greater amberjack ( $n=44$ ) with oocytes in final oocyte maturation (GVM and beyond).

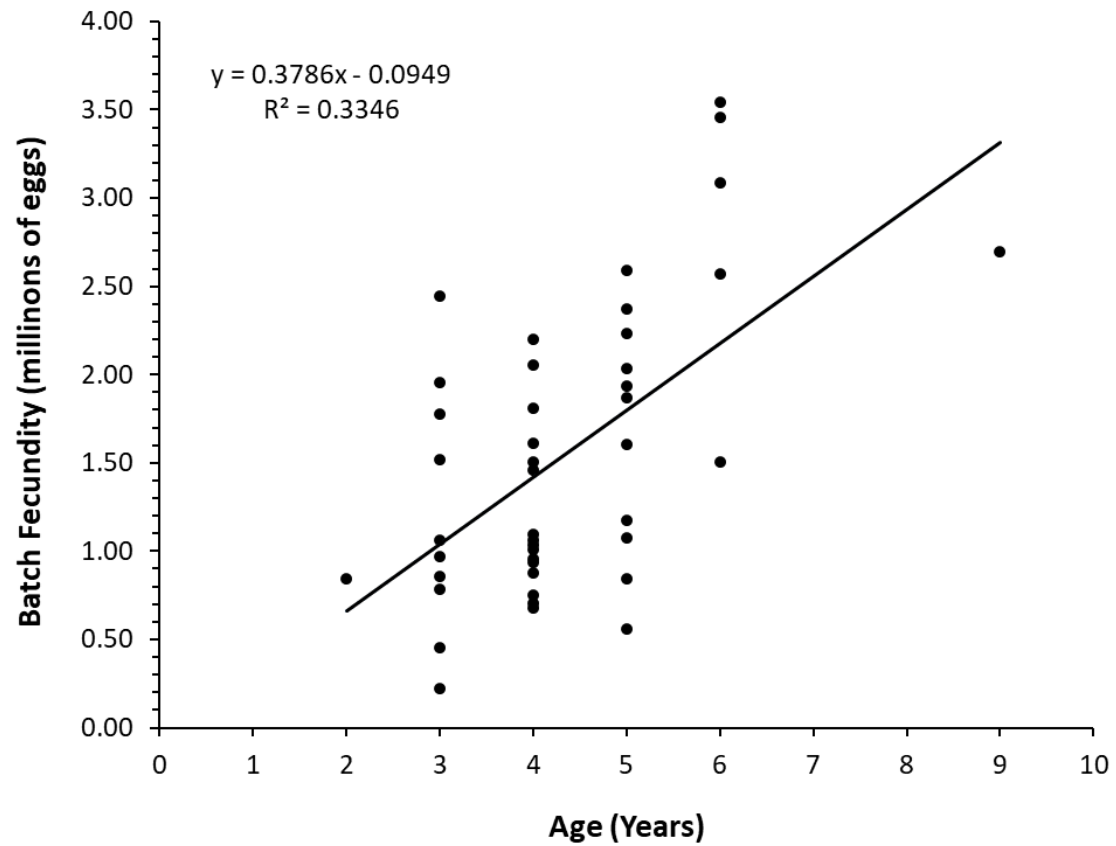


Figure 14. Estimated batch fecundity as a function of age for greater amberjack ( $n=44$ ) with oocytes in final oocyte maturation (GVM and beyond).



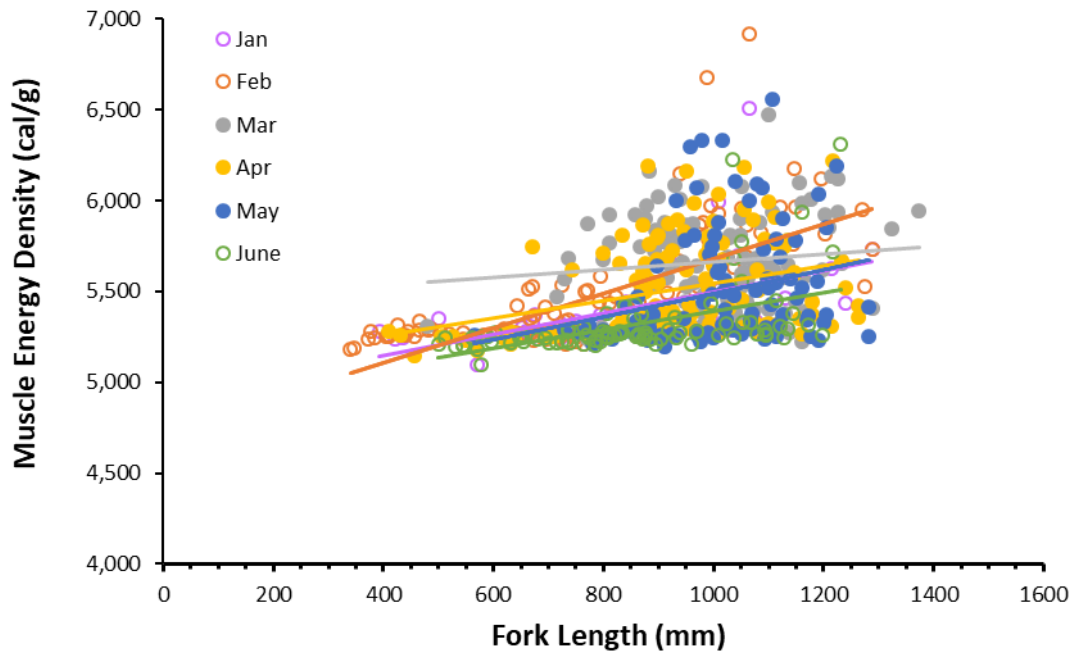


Figure 15. Caloric density (cal/g) of white muscle as a function of female size and month of capture for greater amberjack collected during January through June 2016 and 2017.

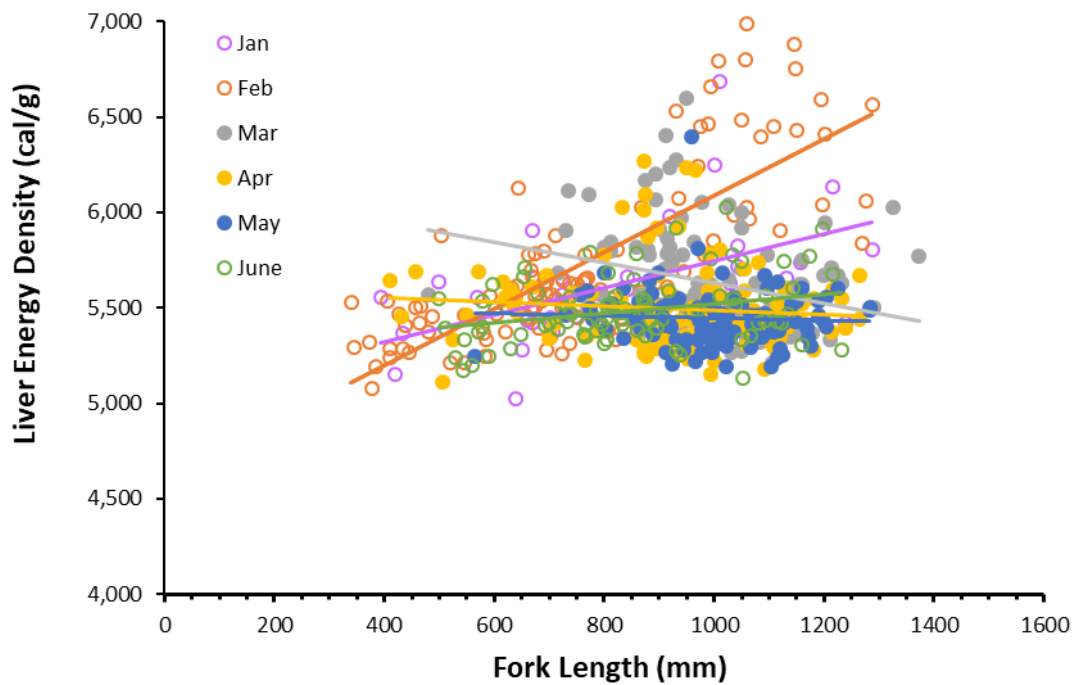


Figure 16. Caloric density (cal/g) of liver as a function of female size and month of capture for greater amberjack collected during January through June 2016 and 2017.

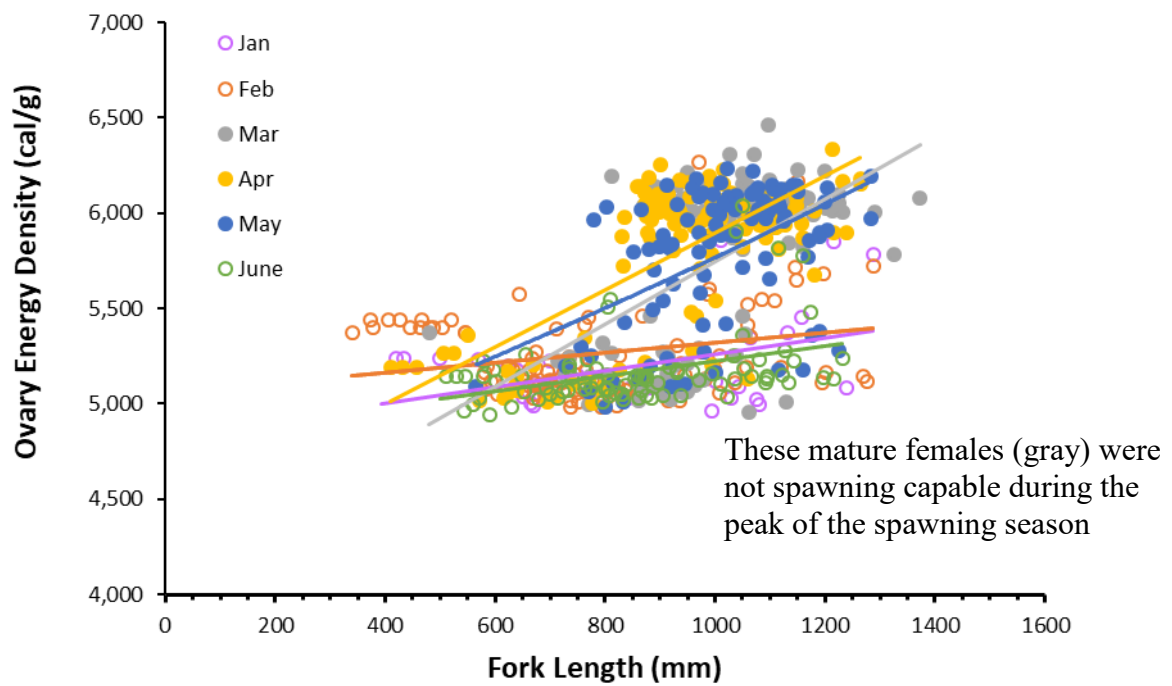


Figure 17. Caloric density (cal/g) of ovarian tissues as a function of female size and month of capture for greater amberjack collected during January through June 2016 and 2017.

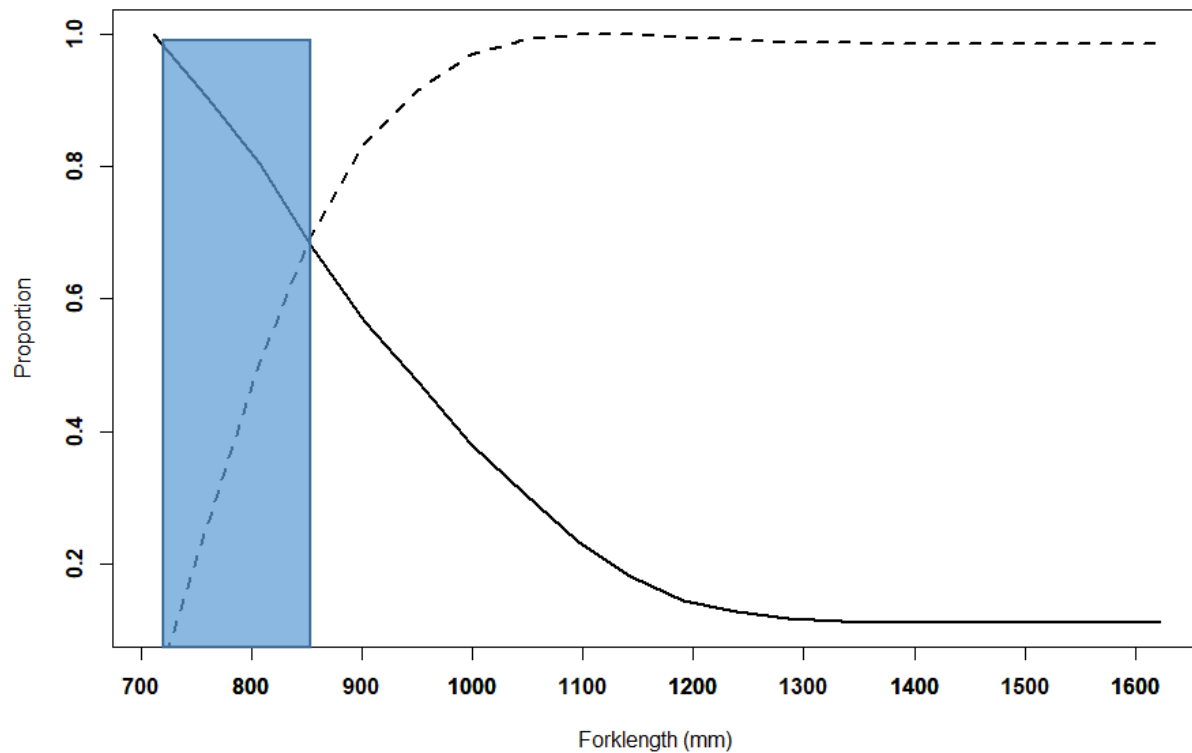


Figure 18A. Effect of implementation of increasing upper slot size on proportion of possible harvest ( ---- ) and proportion of possible large reproductive-sized fish ( — ) with a minimum slot size of 28" (711.2 mm) below the current recreational minimum size limit. Illustrated in blue is the range of a slot from 28" to 34" (711.2 -863 mm). This slot protects most of the reproductive fish, while allowing about 70 % of the population to be potentially harvestable.

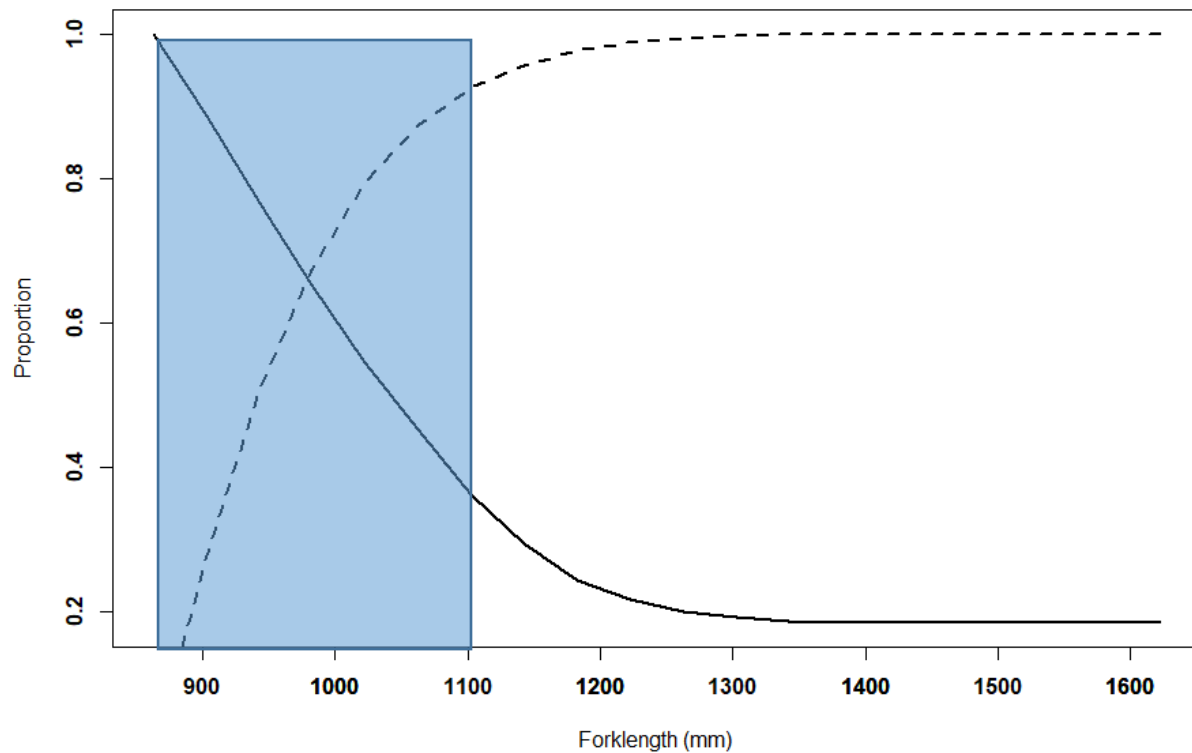


Figure 18B. Effects of implementation of increasing slot size on possible proportion of available harvest in the population ( ----- ) and proportion of large reproductive fish ( ——— ) for the minimum recreational size of 34" (863 mm). Under this scenario, the slot, outlined in blue, maximizes harvest while doing very little to protect the large reproductive fish in the population.

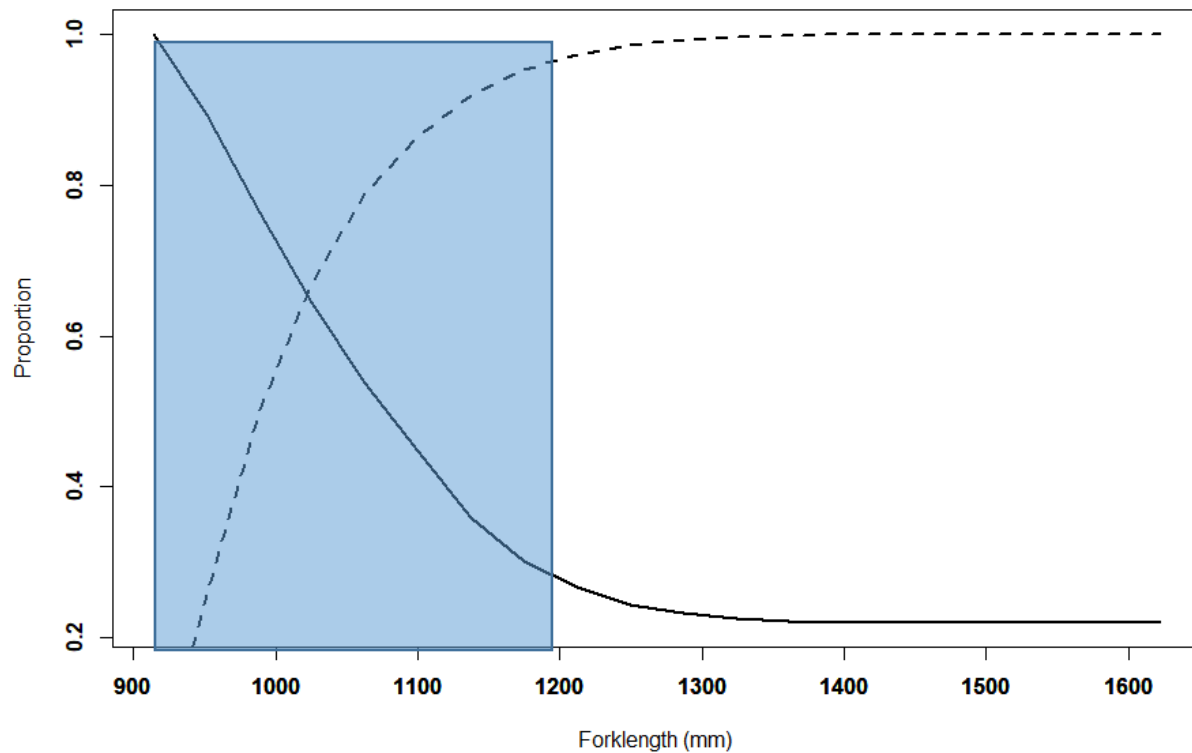


Figure 18C. Effects of implementation of increasing slot size on possible proportion of available harvest in the population ( - - - - - ) and possible proportion of large reproductive fish ( ——— ) for the current minimum commercial size of 36 " (863 mm). Under this scenario, the slot, outlined in blue, performs slightly worse than the slot illustrated in Figure 18B in reducing harvest and protecting the large reproductive fish in the population.