

FIELD BASED NON-LETHAL SEX DETERMINATION  
AND EFFECTS OF SEX RATIO ON POPULATION DYNAMICS OF  
GREATER AMBERJACK, *SERIOLA DUMERILI*

By

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Greater amberjack, *Seriola dumerili*, is a pelagic reef fish that is managed in the US as two separate stocks, the South Atlantic and Gulf of Mexico stocks. The most recent stock assessment for the Gulf of Mexico stock found it to be overfished and undergoing overfishing. Sex-specific spatial distribution and exploitation may contribute to our understanding of the stock's overexploitation because amberjack may be subject to sex-specific mortality resulting from current size regulations, sex-specific growth, and possible skewing of the sex ratio towards one sex or the other in some regions. Current assessments assume a 1:1 sex ratio for the Gulf stock. To explore the potential effect of sex ratio on the Gulf stock's productivity, we first developed a non-lethal method of sex determination to sex greater amberjack released in an ongoing tagging study. The use of external urogenital features allowed for accurate (99.5%, n=194) sexing of greater amberjack over 500 mm FL. Urogenital catheterization provided a means of verifying sex and collecting oocyte samples from females. These samples could be used to determine the relative maturation status of females, although there could be no differentiation made between immature and mature but resting females. Analysis of sex ratios from the non-lethal sexing data and published datasets suggest that the Gulf stock likely has a male to female sex ratio in the

range of 0.5:1 to 1:1 with estimates ranging from 0.4:1 to 1.1:1. To examine the influence of sex ratios on the productivity of the Gulf stock an age-, size-, and sex-structured model was used to model a number of sex ratio scenarios. In general, female-skewing, particularly in the largest size classes, lead to increased stock productivity over the assumed 1:1 sex ratio. Even moderate male-skewing could decrease productivity with some scenarios indicating a stock collapse. These results demonstrate that an incorrect assignment of a presumed sex ratio for the Gulf stock could result in it being mismanaged. It is proposed that a range of realistic sex ratio estimates for this stock should therefore be used in its assessment, rather than continuing to simply assume a sex ratio of 1:1.

## CHAPTER 1 GENERAL INTRODUCTION

Greater amberjack, *Seriola dumerili*, is a pelagic reef species that is found along both the eastern and western Atlantic coasts, in the Mediterranean Sea, and throughout much of the Indian and Pacific Oceans. In the Western Atlantic Ocean, greater amberjack are distributed from Nova Scotia to Brazil, including the Caribbean and Gulf of Mexico (Smith-Vaniz 1984). They tend to congregate around reefs, rocky outcroppings, wrecks, and man-made structures such as oil platforms (Manooch and Potts 1997a, b; Thompson et al. 1999; Harris et al. 2007), which may make them susceptible to overfishing (Beasley 1993). Several extensive studies on the age and growth of greater amberjack have been conducted in the Western Atlantic, but relatively little research has been conducted on reproductive aspects, such as age at maturation, fecundities, and sex ratios. There appear to be no external sexual characters in this species, but comparisons of ages with an adequate sample size for males and females (ages 0-8) have shown that females tend to be larger at age than males (Harris et al. 2007, Murie and Parkyn 2008). This difference in length at age was found to be significant for ages 3, 4, 7, and 9 by Harris et al. (2007) and for ages 2, 4, and 5 by Murie and Parkyn (2008). Beasley (1993) and Thompson et al. (1999) found no difference in the growth rates of males and females from Louisiana but reported that females comprised 72% of the fish over 1 m fork length (FL). Burch (1979) found that beginning with age 4, females were significantly longer than males in southeast Florida. He also noted that the mean monthly FL for females was greater than for males, and that fish greater than 1200 mm were usually females.

A number of studies conducted in the Eastern Atlantic Ocean, mainly in the Mediterranean, have provided information related to various reproductive aspects, but largely focusing on captive culturing efforts (Micale et al. 1993, 1999; Marino et al. 1995a, b; Grau et al.

1996; Kožul et al. 2001; Mylonas et al. 2004). However, as stated previously, there have been few studies in the Western Atlantic focusing on reproduction in this species. Histological examination of gonads from greater amberjack in the Mediterranean has revealed that sexual differentiation is clearly evident in juveniles that are as small as 23 cm standard length (SL) [approximately 245 mm fork length (Uchiyama et al. 1984)] and 4-5 months old (Marino et al. 1995a). It appears that amberjack in the Mediterranean may mature at much larger sizes and older ages than those in the Western Atlantic Ocean (Table 1-1). There are also discrepancies in the maturity estimates obtained for the two studies conducted in the Western Atlantic (Table 1-1). These two studies were conducted on two stocks that are managed separately (NMFS 2006), and that are likely genetically distinct (Gold and Richardson 1998). This may be part of the reason for the disparity in the two sets of estimates, but the differences may also be attributed to the manner in which samples were collected. Murie and Parkyn (2008) noted that Harris et al. (2007) largely targeted a known spawning aggregation with few immature fish being sampled. This would tend to disproportionately represent smaller and younger mature individuals. Murie and Parkyn (2008) also noted, however, that the majority of fish in their study were not sampled from a spawning aggregation and were immature individuals, which may have resulted in some bias towards larger females that were not reproductively active.

A range of sex ratio estimates have been calculated from different regions within the range of this species. Sex ratios (male:female) of 1:1 (Lazzari and Barbera 1989; Micale et al. 1993), 1:2.5 (Thompson et al. 1999), and 1:1.11 (Harris et al. 2007) have been recorded from the Mediterranean, north central Gulf of Mexico, and US South Atlantic coast, respectively. Burch (1979) reported a male to female sex ratio of 1:0.65 from southeast Florida, with males predominating in all months except July, August, and September when females made up

approximately 66% of the catch. Beasley (1993) reported monthly male to female sex ratios of 1:1 to 1:2.06 from the northern Gulf of Mexico off the coast of Louisiana. It has been suggested that in the Gulf of Mexico greater amberjack may show some regional segregation of sexes with females being more prevalent off the coast of Louisiana and males being more prevalent off the western coast Florida, as has been observed for cobia *Rachycentron canadum* (Thompson et al. 1999).

Greater amberjack are a very fecund species with annual fecundity estimates ranging from 18 to 59 million eggs per female, with fecundity varying based on female size (Harris et al. 2007). Greater amberjack can be classified as a multiple-batch, asynchronous spawning fish due to the fact that annual fecundity is indeterminate with all stages of oocytes being present (Murua and Saborido-Rey 2003; Harris et al. 2007). Spawning in greater amberjack varies in time of year based on location and appears to coincide with a temperature increase in the spring (Jerez et al. 2006). Off the Canary Islands, greater amberjack were found to spawn between April and October (Jerez et al. 2006), in the Mediterranean spawning appears to peak between mid-May and mid-July (Marino et al. 1995a), and in south Florida and the Florida Keys peak spawning occurs in April and May (Harris et al. 2007). Thompson et al. (1991) found spawning to occur in May and June off the coast of Louisiana, while Murie and Parkyn (2008) found peak spawning in the Gulf of Mexico to occur in March and April.

Greater amberjack are targeted both recreationally and commercially in the Western Atlantic Ocean. In the United States, greater amberjack are managed as two separate stocks, the US South Atlantic stock and the Gulf of Mexico stock. The boundary for these stocks occurs from approximately the Dry Tortugas through the Florida Keys and to the mainland of Florida (NMFS 2006). The Gulf of Mexico stock is managed by the Gulf of Mexico Fishery

Management Council (GMFMC). Originally, greater amberjack were not included in the Reef Fish Fishery Management Unit (FMU) established by the Reef Fish Fishery Management Plan (FMP), which was implemented in 1984 (Hood 2006). This occurred because greater amberjack were not generally targeted at this time and were considered an incidental catch, and there was also insufficient data available to estimate the maximum sustainable yield (MSY) and optimum yield (OY) for the fishery. However, increases in targeted fishing for this species and the resulting effects on the stock size have led to a number of regulatory measures, which are summarized in Table 1-2. In 1996, an assessment was conducted for the Gulf stock (McClellan and Cummings 1996), but it was deemed too imprecise to specify an acceptable biological catch or set a total allowable catch (TAC) (Hood 2006). The stock was re-assessed in 2000 by Turner et al. (2000). The four most likely model runs from this assessment indicated that the stock was overfished. A status of overfished indicates a stock condition in which the current biomass is less than the Minimum Stock Size Threshold ( $MSST = (1-M) \cdot B_{MSY}$ ), where  $M$  is the natural mortality rate and  $B_{MSY}$  is the biomass capable of producing MSY. Two of these four runs also indicated that the stock was undergoing overfishing, a condition in which the current exploitation rate ( $F_{current}$ ) exceeds the exploitation rate that would produce MSY ( $F_{MSY}$ ) (NMFS 2006). In 2001, the GMFMC was notified by the National Marine Fisheries Service that the Gulf stock was overfished. This resulted in Secretarial Amendment 2, which contained biological reference points, status determination criteria, and a 10-yr rebuilding plan (Hood 2006). The most recent stock assessment for greater amberjack in the Gulf of Mexico (NMFS 2006) indicated that this fishery remains overfished and is undergoing overfishing. This stock assessment and further concerns about the Gulf stock's status have led to further regulation of the Gulf stock (Table 1-2).

The 2006 stock assessment (NMFS 2006) was based on the best available data, but there was still a substantial lack of adequate information available, which resulted in the use of surrogate parameters from the US South Atlantic stock and proxies, such as weight-at-maturity as a proxy for fecundity. Some of these data gaps, such as information on age and growth, have been recently acquired (Murie and Parkyn 2008). Many aspects of reproductive biology of greater amberjack in the Gulf of Mexico, however, are lacking, yet are critical to understanding their sustainability. Reproductive seasonality and fecundity are currently being studied (D. Murie et al., University of Florida, unpublished data), but other reproductive parameters, such as sex ratio, are unknown. Without information on the sex ratio for the Gulf stock of amberjack it must be assumed that it is 1:1, as was the case in the current stock assessment, although it was unknown how this would influence the population dynamics of greater amberjack. Regional segregation by sex, as suggested by Thompson et al. (1999), may result in regional skewing of sex ratios and hence disproportionate representation of one sex or the other in the catches from a particular region. There is also a potential for a disproportionate representation of females in the harvested catch due to the faster growth of females in comparison to males and the minimum size limits placed on the fisheries (i.e. sex selectivity by the fishery). This may be particularly true in the commercial fishery where its minimum size limit of 914 mm fork length (FL) would result in a majority of very large fish being harvested, which would be mostly females. Disproportionate catches of one sex over the other could lead to an alteration of the overall sex ratio, which may impact the population dynamics of the stock due to possible egg or sperm limitation arising from low numbers of mature individuals of a particular sex (Huntsman and Schaaf 1994; Armsworth 2001; Alonzo and Mangel 2004, 2005; Heppell et al. 2006; Molloy et al. 2007; Alonzo et al. 2008).

Obtaining data on the sex of greater amberjack landed in both commercial and recreational fisheries may be difficult and potentially biased. In the commercial fishery, fish are generally brought to port gutted, making sexing by examination of the gonads impossible. In addition, port sampling of the recreational fishery sector generally only samples a small portion of the landed catch, which may represent only a small fraction of the total catch due to size regulations and mandatory discarding of under-sized fish. If there is sex-selectivity in the landings of a fishery, sex ratios derived from fisheries data may also be biased towards the selected sex, while the sex ratio of the remaining, non-harvested population may be different and possibly becoming skewed toward the opposite sex. The development of a non-lethal sexing method for greater amberjack would allow for an alternative method of estimating sex ratios. Such a method could be applied in the field by researchers or onboard fishery observers to determine the sex of the entire catch, including discards, rather than simply obtaining sex information by sampling a fraction of the landed catch.

The overall goal of this study was to examine the influence of sex ratios on the population dynamics of greater amberjack in the Gulf of Mexico. Specific objectives included: 1) development and validation of a non-lethal method to sex fish by external examination of the urogenital area; 2) evaluation of general maturation status of females via urogenital catheterization; 3) analysis of sex ratios based on published datasets and from field based non-lethal sexing; and 4) assessment of the impact of a range of potential sex ratios on the productivity of Gulf of Mexico greater amberjack. A non-lethal method of sexing greater amberjack was developed based on differences associated with the genital and urinary pores apparent between males and females, and validation of this method was obtained through the expression of milt, the collection of gonadal material (milt, oocytes, ovarian lamellae) via

urogenital catheterization, and sacrificed individuals (Chapter 2). Application of this method to fish captured in an ongoing tag-and-release study on greater amberjack in the Gulf of Mexico allowed for an alternative means of estimating overall sex ratios, as well as sex ratios based on size. Sex ratio estimates were also obtained from published dataset (Murie and Parkyn 2008), and these two sets of sex ratio estimates, as well as previously published sex ratios from the region, were used to develop a range of potential sex ratio scenarios (Chapter 2). A size, age, and sex based population model was then used to assess the potential impacts on both male and female reproductive potential under each of these sex ratio scenarios (Chapter 3). In conclusion, Chapter 4 summarized the new information obtained during the course of this study, including the use of external urogenital features and urogenital catheterization to sex and stage female greater amberjack, the possible sex ratio trends for the Gulf stock, and the potential effects of sex ratios differing from 1:1 on the productivity of the Gulf stock.

Table 1-1. Age and size at maturity estimates for greater amberjack from different regions of the eastern and western Atlantic Ocean.

Sex	<u>First mature</u>		<u>50% mature</u>		<u>Last immature</u>		Region	Reference
	Age	Size (mm FL)	Age	Size (mm FL)	Age	Size (mm FL)		
Male	2	650 <sup>a</sup>	.	1150 <sup>a</sup>	.	.	Mediterranean	Marino et al. 1995b
	1	464	.	644	5	755	US South Atlantic	Harris et al. 2007
Female	3	850 <sup>a</sup>	.	1200 <sup>a</sup>	.	.	Mediterranean	Marino et al. 1995b
	1	514	1.3	733	5	826	US South Atlantic	Harris et al. 2007
	1	501	3-4	850-900	6	.	Gulf of Mexico	Murie and Parkyn 2008
Combined	.	.	3	.	4 <sup>b</sup>	.	Gulf of Mexico	Thompson et al. 1991

<sup>a</sup> Standard length was converted to forklength (FL) using equation provided by Uhiyama et al. (1984).

<sup>b</sup> All fish examined were mature by this age.

Table 1-2. History of management of the Gulf of Mexico greater amberjack stock (Hood 2006; NMFS 2008, 2009 a, b, 2010 a, b).

Year	Amendment	Regulations	Justification
1990	Amendment 1 to the Reef Fish FMP	added to Reef Fish FMU; 28 in (711 mm) FL minimum size (recreational); 3 fish/angler bag limit (recreational); 36 in (914 mm) FL minimum size (commercial)	protect stock and prevent overfishing; landings declining since 1982; targeted more as other target species declined in abundance
1997	Amendment 12 to the Reef Fish FMP	1 fish/angler bag limit (recreational)	anecdotal information from fishermen that the average size of fish and abundance had decreased
1998	.	March-May closure (commercial)	concerns about abundance continuing to decline, reduce commercial catch by amount similar to recreational bag limit reduction
2001	Secretarial Amendment 2	biological reference points, status determination criteria, and 10-yr rebuilding plan that would limit harvest for 3 year intervals: 2.9 million lbs (1.3 million kg) for 2003-2005 5.2 million lbs (2.4 million kg) for 2006-2008 7.0 million lbs (3.2 million kg) for 2009-2011 7.9 million lbs (3.6 million kg) for 2012	NMFS declared the stock overfished in January 2001
2008	Amendment 30A to the Reef Fish FMP	30 in (762 mm) FL minimum size (recreational); zero bag limit for captains and crew of for-hire vessels; 0.503 million lbs (0.228 million kg) commercial quota; 1.368 million lbs (0.621 million kg) recreational quota	2006 stock assessment found stock to be overfished and undergoing overfishing
2009	.	temporary recreational closure (Oct 24-Dec 31)	quota met or exceeded
2009	.	temporary commercial closure (Nov 7-Dec 31)	quota met or exceeded
2010	.	0.373 million lbs (0.169 million kg) commercial quota	reduction in quota for 2009 overage
2010	.	1.243 million lbs (0.564 million kg) recreational quota	reduction in quota for 2009 overage
2010	.	temporary commercial closure (Oct 28-Dec 31)	quota met or exceeded

## CHAPTER 2 NON-LETHAL SEX DETERMINATION AND SEX RATIOS OF GREATER AMBERJACK

### **Overview**

An important aspect for the management of fish species and successful aquaculture programs is the ability to accurately determine the sex and maturation status of individuals. This information can be used to determine parameters in the assessment of wild populations, such as sex ratios, age and size at maturation, and potential fecundity (Blythe et al. 1994; Martin-Robichaud and Rommens 2001; Whiteman et al. 2005; Swenson et al. 2007). For aquaculture programs to be successful the sex and maturity of individuals is needed to maintain proper sex ratios and to select fish to maintain for broodstock, as well as to aid in timing of induced spawning or stripping of eggs (Martin et al. 1983; Shields et al. 1993; Blythe et al. 1994; Martin-Robichaud and Rommens 2001; Moghim et al. 2002; Alam and Nakamura 2008; Newman et al. 2008). However, for fish showing little (i.e. differential growth in sexes) or no sexual dimorphisms, this information is traditionally gained by sacrificing the fish and performing post-mortem dissections for fisheries or through gonadal biopsies in aquaculture facilities (Martin-Robichaud and Rommens 2001; Swenson et al. 2007). Developing a means of determining the sex and maturity of individuals without sacrificing them is of particular interest to the management of endangered or threatened species where it is undesirable to sacrifice any individuals (Blythe et al. 1994; Moghim et al. 2002; Colombo et al. 2004; Bryan et al. 2007), for non-lethal tag and release studies (St-Pierre 1992), and to aquaculture programs where this information would maximize production and profit by maintaining appropriate sex ratios without sacrificing broodstock (Martin et al. 1983; Reimers et al. 1987; Mattson 1991; Karlsen and Holm 1994; Blythe et al. 1994; Matsubara et al. 1999; Moghim et al. 2002; Alam and Nakamura 2008). As discussed in Chapter 1, the development of a non-lethal sexing technique for greater

amberjack would provide an alternative means to obtain information relating to reproduction, such as calculating sex ratios, which often rely on port sampled fish that may not represent the entire population.

Several non-lethal methods have been developed to assess the sex and maturity of fish with varying degrees of success, including: 1) analyzing steroid, hormone, and protein levels; 2) determining sex chromosomes; 3) palpating the gonad; 4) surgical observation and biopsy; 5) endoscopy; 6) ultrasonography; 7) urogenital catheterization; and 8) examining external urogenital features.

### **Steroid, Hormone, and Protein Levels and Sex Chromosomes**

Radioimmunoassays of several blood plasma indicators have been investigated to determine sex and maturation stage of fish. Among the blood plasma indicators used are the steroids 11-ketotestosterone, estradiol, and testosterone. In some cases these indicators can also be found in muscle fiber (Heppell and Sullivan 2000). This method has been used to determine the sex and maturation status of a number of species (Sangalang et al. 1978; Johnson and Casillas 1991; Heppell and Sullivan 2000; Webb et al. 2002; Evans et al. 2004; Feist et al. 2004) (Table 2-1). The detection of the female-specific protein vitellogenin in the blood or skin mucus has also been used to determine the sex of some species (Le Bail and Breton 1981; Gordon et al. 1984; Takemura et al. 1996; Heppell and Sullivan 1999) (Table 2-1). Vitellogenin can be detected through the use of immunoagglutination or enzyme-linked immunosorbent assay (ELISA).

Methods including plasma lipophosphoprotein analysis, plasma vitellogenin concentrations, immunoagglutination, and radioimmunoassay of blood steroid and hormone levels can be successful in identifying the sex of individuals, but they have several draw backs. The collection of blood and/or oocytes involved with several of these methods may cause

excessive stress, introduce pathogens, and delay or prevent ovulation (Blythe et al. 1994; Moghim et al. 2002). These methods may be costly, do not provide immediate results, and may only be accurate if fish are mature or only during certain periods during the reproductive cycle (Martin et al. 1983; Blythe et al. 1994; Martin-Robichaud and Rommens 2001; Moghim et al. 2002; Colombo et al. 2004), and in the case of blood plasma indicators, the technique is species-specific with a baseline needed for each species examined (Colombo et al. 2004).

Analysis of genetic samples for sex chromosomes can also be used to sex some fish species. However, a number of species, including greater amberjack, lack sex chromosomes (Sola et al. 1997).

### **Palpation**

Palpation of the gonad by insertion of the finger through the mouth and into the stomach has allowed for accurate sexing of several small salmonid species (Kano 2005) (Table 2-1). This method is relatively non-invasive but is limited by the size and species of fish that can be examined. In small fish a finger may be too large to insert into the mouth, while in large fish a finger may not be long enough to reach the stomach and therefore cannot be used to feel the gonads through the wall of the stomach. Also, species with pharyngeal teeth, such as carp *Cyprinus carpio*, or species with other types of teeth that could potentially injure the investigator, cannot be sexed with this method (Kano 2005).

### **Surgical Observation and Biopsy**

Performing surgeries on live fish to directly examine the gonads and to remove a gonadal sample for biopsy has been used to determine the sex and maturation status of several species (Ritchie 1965, Alam and Nakamura 2008) (Table 2-1). In some cases this method has become a standard practice for determining the sex and maturity of fish, most notably in sturgeon (Johnson and Casillas 1991; Kynard and Kieffer 2002; Webb et al. 2002; Colombo et al. 2004; Feist et al.

2004) Alam and Nakamura (2008) performed surgery on honeycomb grouper *Epinephelus merra* in a lab setting to extract gonadal tissue for biopsy. This method resulted in 100% correct identification of sex and maturity with no serious infections or deaths resulting, and apparently no lasting damage to the gonads (Table 2-1). In some species, gonadal samples for biopsy may be obtained by insertion of forceps into the urogenital pore. This method has been used on striped bass *Morone saxatilis* with an accuracy of 95%, but some fish had gonadal wounds that had not healed at autopsy (Ritchie 1965) (Table 2-1).

Gonadal biopsies performed on live fish can result in very accurate identification of sex in many cases, but it may prove less accurate for some species and for immature individuals where collection of gonadal tissue is difficult (Johnson and Casillas 1991; Webb et al. 2002). Accuracies of gonadal biopsies may also be reduced when adipose tissue or tissue from other organs is mistakenly collected instead of gonadal tissue (Webb et al. 2002). Biopsies on live fish are also invasive and have the potential to cause trauma to the gonad (Moccia et al 1984; Mattson 1991; Johnson and Casillas 1991; Kynard and Kieffer 2002; Webb et al. 2002; Colombo et al. 2002). This technique may be difficult, if not impossible, to perform on a moving boat in the field without causing injury to the fish. Also, an anesthetic that is approved for immediate release of fish must be used for studies done in the field, especially in fish that may be consumed, and fish must be fully revived before they are released to reduce post-release predation (Columbia Basin Fish and Wildlife Authority 1999; Coyle et al. 2004; Kahn and Mohead 2010).

### **Endoscopy**

Endoscopy involves the insertion of an endoscope either through the urogenital pore or a small incision in the abdomen, where the gonads are viewed either through the urogenital duct or directly. An otoscope, a device normally used for examining the interior of the human ear, was

one of the first devices to be used as an endoscope for determining sex in fish. It allowed largemouth bass *Micropterus salmoides* to be sexed with high accuracy (Driscoll 1969) (Table 2-1). Typical devices used for endoscopy of fish are borescopes, rigid or flexible devices with an eyepiece on one end and an objective lens on the other linked by a relay optical system surrounded by optical fibers, or endoscopes, which have the same components as a borescope but also contain a channel for the insertion of instruments or manipulators. Both devices can be used with a video-imaging system. Endoscopy has been used to successfully determine the sex and maturational status of a number of species (Moccia et al. 1984; Ortenburger et al. 1996; Kynard and Kieffer 2002; Wildhaber et al. 2005; Bryan et al. 2007; Swenson et al. 2007) (Table 2-1).

Examination of gonads by endoscopy provides immediate results and in at least some cases can predict sex accurately throughout the reproductive cycle. However, this technique requires some expertise and a detailed knowledge of the internal anatomy of the body cavity of the fish species being examined. In addition, this is still an invasive method, particularly when an incision needs to be made. The stress involved, along with the loss of epidermal mucus, drying of skin, and damage to internal organs could potentially lead to mortality (Swenson et al. 2007). There is also the potential for later complications, such as an incision reopening or infection (Swenson et al. 2007). Endoscopy can also be a relatively lengthy process, from the time a fish is anesthetized until the time it is revived can range from 2 to 10 minutes on average (Moccia et al. 1984; Ortenburger et al. 1996; Swenson et al. 2007). Video-endoscopy has been successfully used in the field, but it has only been performed from a shore base. As with gonadal biopsies, this technique may be difficult, if not impossible, to perform on a moving boat without causing injury to the fish. In addition, an anesthetic is often needed and would have similar limitations as with surgical biopsies.

## Ultrasound

Ultrasound imaging has been used to accurately determine the sex of a number of fish species throughout much of their various reproductive cycles (Martin et al. 1983; Reimers et al. 1987; Mattson 1991; Bonar et al. 1989; Blythe et al. 1994; Karlsen and Holm 1994; Matsubara et al. 1999; Martin-Robichaud and Rommens 2001; Moghim et al. 2002; Burtle et al. 2003; Colombo et al. 2004; Wildhaber et al. 2005; Whiteman et al. 2005; Newman et al. 2008) (Table 2-1). It has also been used to determine the maturational status of a number of species (Reimers et al. 1987; Shields et al. 1993; Blythe et al. 1994; Martin-Robichaud and Rommens 2001; Moghim et al. 2002; Burtle et al. 2003; Evans et al. 2004; Bryan et al. 2005, 2007; Newman et al. 2008) (Table 2-1). Gonad diameter can be estimated in some cases, which could allow for the estimation of a gonadosomatic index (size of gonads relative to the fish size) or the development of a similar reproductive index (Mattson 1991; Newman et al. 2008).

Pulse-echo acoustic microscopy, which is essentially an adaptation of ultrasound imaging using a focusing lens to concentrate the high frequency ultrasound to produce high resolution images, has been shown to be an effective means of sexing larval sea lampreys *Petromyzon marinus* (Maeva et al. 2004). This technology would likely produce similar results in larval or small juvenile fish of other species and has the potential to be adapted to field use.

Ultrasound technology has also been used in several cases to determine batch fecundity estimates. Fecundity estimates for striped bass, which were comparable to fecundity estimates obtained via traditional methods in previous studies, have been determined by estimating ovary volume from ultrasound images and collecting oocyte samples via a catheter (Will et al. 2002; Jennings et al. 2005). Fecundity estimates for red hind *Epinephalus guttatus* have also been made using this method (Whiteman et al. 2005), which fell within the ranges of other published estimates for this species. Fecundity estimates for Neosho madtoms *Noturus placidus* based on

oocyte and ovary volumes calculated from ultrasound images were found to be similar to those of other madtom species (Bryan et al. 2005). The fecundity of shovelnose sturgeon *Scaphirhynchus platyrhynchus* was determined using ovary volumes calculated from ultrasound images in combination with oocyte volumes calculated from ultrasound images and oocyte samples (Bryan et al. 2007).

Ultrasound images can provide rapid and immediate results, and the method is non-invasive. However, as with endoscopy, considerable expertise in the use of the equipment and a detailed knowledge of the internal anatomy of the body cavity of the species being examined is required. In some cases sex can only be determined by the presence or absence of ovaries as the testes can be difficult to discern. The accuracy of ultrasound tends to be lessened in immature and post-spawned individuals. The size, shape, and composition of scales and the thickness of the abdominal wall may also influence the accuracy of this method. Anesthesia is not necessary, but may be desirable for producing better image quality. Ultrasound can also be particularly cost prohibitive in obtaining a unit with the proper resolution needed for accurate determination of sex and maturation status.

### **Urogenital Catheterization**

Urogenital catheterization involves the insertion of a small-diameter glass or plastic catheter into the urogenital or genital pore to collect a gonadal sample. The sample is either collected by mouth suction or suction via syringe as the catheter is slowly pulled back out of the gonad. The diameter of the catheter used generally depends on the size of the fish, urogenital pore, and eggs to be sampled. The relatively small diameter of the vas deferens can prevent catheterization in males of some species (Ross 1984; Benz and Jacobs 1986). Determination of sex and maturational status has been obtained with the use of urogenital catheters on a number of species (Shehadeh et al. 1972; McEvoy 1983; Ross 1984; Garcia 1989; Bailey and Cole 1999;

Mackie 2000, 2003; Alvarez- Lajonchère et al. 2001; Coward and Bromage 2001; Kožul et al. 2001; Asturiano et al. 2003; Marino et al 2003; Mylonas et al. 2003, 2004; Ferraz et al. 2004) (Table 2-1). Sexing data may not always be reliable as it is often difficult to extract oocyte samples via catheterization from immature and non-reproductive females (Mackie 2000, 2003). Several studies have shown that oocyte samples obtained via catheterization are not significantly different from samples taken directly from ovaries of the same fish post-mortem, and these samples are representative of the whole ovary in species showing synchrony in ovarian development (Shehadeh et al. 1972; Garcia 1989; Alvarez-Lajonchère et al. 2001; Coward and Bromage 2001; Ferraz et al. 2004).

Urogenital catheterization may provide information on sex and maturity; however, in immature and non-reproductive fish, as well as males in some species, it may be difficult to obtain samples. This is a relatively rapid and inexpensive sampling technique that requires little training. It can also be easily used in the field. Few deleterious effects of catheterization have been reported in studies using this method. However, it is invasive and is thought to cause stress, harm, and even direct mortality (Blythe et al. 1994; Martin-Robichaud and Rommens 2001; Kynard and Kieffer 2002; Moghim et al. 2002; Newman et al. 2008).

### **External Urogenital Features**

Several methods of evaluating the sex of fish externally have been developed involving direct examination of the urogenital area. These methods have been applied to a variety of fish species with varying degrees of success. Some of these methods are relatively inaccurate, while others have consistently shown accuracies comparable to the methods discussed above (Table 2-1). One of the simplest and most obvious ways to sex fish externally is through the expression of milt or eggs by pressure applied to the abdomen. This method is restricted to the time immediately surrounding spawning (Parker 1971; Casselman 1974) and generally has to be

combined with some other external method, such as the presence of a swollen vent, to provide high accuracies (Snow 1963; Benz and Jacobs 1986). In some species this method may not be reliable because dense abdominal musculature may prevent the expression of milt and eggs (Mylonas et al. 2004).

Other external methods of sexing fish rely on finding some morphological difference in the urogenital area of the fish. In some of these methods, sexing is based on morphological features in the area surrounding the urogenital pore(s). The sex of largemouth bass can be determined by the shape of the scaleless area surrounding the urogenital openings (Parker 1971). In males this area is nearly circular in shape, while in females it is elliptical or pear-shaped. This method did not appear to be influenced by size but may have limitations associated with size and condition of the fish (i.e. distended abdomen due to food and/or roe or concave abdomen due to emaciation) (Parker 1971; Manns and Whiteside 1979). The presence of a swollen reddish genital papilla in female largemouth bass can also be used to sex largemouth bass, but this method is only seasonally accurate (Benz and Jacobs 1986). In adult rock bass *Ambloplites rupestris* the shape and color of the urogenital papillae, the shape of the scaleless area surrounding the urogenital and anal pores, and the distance between the urogenital and anal openings can be used as an indication of sex (Noltie 1985). In males, the urogenital papilla is pointed and black at the tip, the scaleless area is circular, and the relative distance between the urogenital and anal pores is smaller than in females. In females, the urogenital papilla is blunt, swollen, and red at the tip and the scaleless area is oval in shape. Some of the characters associated with this method are related to spawning, and thus accuracy may be reduced when these characters are not fully developed. The appearance of the urogenital area of northern pike *Esox lucius* has been used to sex males and females with a high degree of accuracy regardless of

maturity or season (Casselman 1974). In females, the area between the anus and urogenital pore contains many longitudinal folds, while males have only up to three slight grooves in this area. The area is often raised above the surrounding tissue in females, especially near spawning, while in males it is almost always level with the surrounding tissue.

Several methods use the shape and/or number of urogenital pore(s) to determine sex. Mature bluegill *Lepomis macrochirus* were sexed with 100% accuracy based on the shape of the urogenital pore and the appearance of the surrounding tissue (McComish 1968). In females, the urogenital pore resembles a doughnut-like ring surrounded by pink, fleshy tissue. The male urogenital pore never has a ring-like appearance, and instead it is small and funnel shaped, and is surrounded by little, or no, pink fleshy tissue. This method appeared to only be dependent on the maturity of the fish and was not influenced by season. The depth and angle of penetration of a probe into the urogenital pore of largemouth bass can yield high sexing accuracies (Benz and Jacobs 1986). In males the penetration of a probe is shallow and perpendicular to the ventral surface of the fish, while in females the probe penetration is deeper and oblique to the ventral surface of the fish. Sigler (1948) found that white bass *Morone chrysops* could be sexed externally based upon the number of urogenital pores. In males there was a single urogenital pore, while in females there were separate genital and urinary pores. A small pit just posterior to the urogenital pore in males could be mistaken as a urinary pore, but the use of a blunt probe eliminated this potential source of error. In bocaccio *Sebastes paucispinis* the opening for the genital and urinary duct occur on a common urinary papilla in males, while in females the genital opening is present between the anus and the urinary papilla (Moser 1967). This anatomical trait was successfully used to sex both quillback rockfish *S. maliger* and copper rockfish *S. caurinus* in the field, underwater at 25 m depth (Murie 1991). Channel catfish *Ictalurus punctatus* can be

sexed with an accuracy of 99%, regardless of body size and maturity, based on the number of urogenital pores (Norton et al. 1976). In males there is a single urogenital pore, while females have a genital pore and a urinary pore separated externally by a septum. In Pacific halibut *Hippoglossus stenolepis* the shape of the cloaca could be used to accurately sex individuals 52 cm or larger with an accuracy of 98% (St-Pierre 1992). In females, the cloaca is a small cone-shaped projection with the terminal end angled towards the anal fin. Males have a cloaca that is also cone-shaped, but it is truncated and oriented nearly perpendicular to the body. In halibut, the genital vent of males is much larger than that of females. The inability to sex smaller individuals is partially attributed to the smaller size of the cloaca in these individuals making it difficult or impossible to view with the naked eye. The sex of several North American sturgeon species has been determined in live individuals based on the shape of the urogenital opening (Vecsei et al. 2003). In males, the urogenital opening is in the shape of the letter Y, while in females it is in the shape of the letter O. This method was unreliable in dead specimens as the rectum is generally prolapsed causing the urogenital opening to protrude. In the aquaculture of various tilapia species the sexes are separated based on the number of urogenital pores (Rakocy and McGinty 1989; Popma and Masser 1999). In males there is only one urogenital pore, while in females there are separate genital and urinary pores. Applying dye to the urogenital region can increase the accuracy of sexing and may allow for the sexing of smaller individuals (Rakocy and McGinty 1989; Popma and Masser 1999).

External sexing methods based on morphological differences of the urogenital region generally result in high accuracies, and in many cases may not be restricted by size, maturity, or season. Several of these methods are completely non-invasive, while others are only minimally invasive requiring a probe to be inserted into the urogenital pore(s). These procedures are quick,

do not require anesthesia, and require little training, which are all desirable for application in the field. However, maturation status cannot be obtained through external sexing methods other than through stripping milt and eggs by applying pressure to the abdomen.

### **Method of Choice for Greater Amberjack**

Using previous studies of other species as a guide, a non-lethal sexing methodology for field-based sexing of greater amberjack needed to meet several criteria, including being minimally invasive and having a high accuracy at various sizes/ages and stages of maturity. Methods requiring minimal training, having low cost, and providing immediate results were also desirable. Based on these criteria, ultrasonography, external examination of the urogenital pores, and urogenital catheterization appeared to be appropriate as methods to investigate in greater amberjack.

Preliminary attempts were made to sex greater amberjack with ultrasonography using a portable ultrasound unit (Carewell CUS-3000 with a LU2-2/7.5MHz linear array probe) loaned by C. Koenig (Florida State University). However, images of the organs in the body cavity were not clear enough to discern the gonads. This lack of clarity applied to amberjack specifically since comparable ultrasound of a striped bass produced images that were clearer, although still not conclusive. This particular ultrasound unit may not have had a high enough resolution for sexing of amberjack due to the fish's thick abdominal musculature. Portable ultrasound units with higher resolution are available, but were cost prohibitive. In addition, the high content of guanine crystals in the thick epidermal tissue, peritoneum, and gas bladder of amberjack may also have affected the quality of the image (D. Parkyn, University of Florida, personal communication). The use of ultrasonography was therefore not considered a viable option for sexing greater amberjack.

The use of urogenital catheterization was also appealing due to its minimal cost, minimal invasiveness, lack of anesthesia, and lack of extensive training. However, it may be largely limited by size and maturity of the fish being examined. Using external urogenital features for non-lethal sexing also has a number of desirable characteristics including: minimal invasiveness, minimal training, relatively rapid to perform, provides immediate results, and not being cost prohibitive. The major potential drawbacks to this method are the potential for size or maturation limitations and the lack of information on maturational status. However, a number of species sexed via this method have shown minimal size and/or maturation limitations, and combining this method with urogenital catheterization could provide some information on maturation status. In addition, the use of urogenital catheterization has been successfully used on mature greater amberjack (Kožul et al. 2001; Mylonas et al. 2004).

The overall goal was to develop a non-lethal method of sexing greater amberjack in the field, with specific objectives: 1) to determine the accuracy of using external urogenital features to sex greater amberjack; 2) to examine the utility of using urogenital catheterization as a method of determining the gonadal maturation of female greater amberjack; and 3) to directly apply non-lethal sexing of field-sampled greater amberjack as an alternative method of determining sex ratios compared to estimates obtained from data collected in previous studies using lethal methods.

## **Methods**

### **Sex Differentiation of Urogenital Pores**

Initially, 8 (6 males and 2 females) greater amberjack were collected as part of an ongoing tagging study (D. Murie and D. Parkyn, University of Florida, unpublished data) in November 2008 to January 2009, and were sacrificed to examine their urogenital regions for the presence of morphological differences in the urogenital pores and surrounding tissues. Additional

observations were made on 3 individuals (1 male and 2 females) that were sexed in the field and sacrificed for validation in March 2009. A blunt probe was used to locate the anus and urogenital pore(s), and differences in the spacing, location, and general appearance of the urogenital pore(s) and surrounding tissues was noted.

### **Field-based Sex Identification using Urogenital Pores**

To apply the external sexing of amberjack to field samples, and determine the accuracy of the method, amberjack were sexed in the field during tagging trips in March 2009, April 2009, May 2009, November 2009, March 2010, April 2010, and June 2010. Fish were caught with hook and line and bandit fishing gear off the coast of Little Torch Key, Madiera Beach, Suwannee, and Apalachicola, FL, and Grande Isle, LA. Fish were measured for fork length (FL, nearest mm), tagged below the anterior portion of the second dorsal fin with a dart tag, and 2 to 3 fin rays between rays 3 and 6 of the left pectoral fin were removed for ageing and genetic analysis as part of the tagging study. Fish were then sexed by examining external features of their urogenital region. To do this, a blunt probe was used to find both the genital and urinary pore and then the fish was scored as a male or female based on the location of each pore in relation to the other, and the appearance of the pores and surrounding tissue, using the sexing differentiation criteria.

### **Accuracy of Sex Determination**

Validation of the field-based sex identification was obtained through urogenital catheterization, the expression of milt on insertion of a blunt probe into the genital pore or through abdominal pressure, and sacrificed individuals. Fish that were captured with oocytes extruded out the genital pore or that were freely flowing milt were not used to determine accuracy of the external sexing method.

Sexing and catheterization of fish was performed while they were placed on their side on a measuring board. During the initial use of this method in the field on live fish, it was discovered that when locating and examining the genital pore with a blunt probe, mature male fish would often express milt if the probe was inserted a few mm into the genital pore. After finding this, a blunt probe was inserted into the genital pore of all fish large enough to do so in an attempt to express milt as a means of sex verification.

Urogenital catheterization was attempted on all females that appeared to be reproductively active, as well as randomly on both males (that did not express milt) and females of various sizes. The catheter used consisted of a 3 ml Luer-Lok tip disposable syringe and plastic microbore tubing with the following specifications: inner diameter of 0.76 mm, outer diameter of 2.23 mm, wall thickness of 0.76 mm, and length of ~20 cm (Figure 2-1). The tubing was attached to the syringe via a 1/16" (1.6 mm) ID female Luer-thread style to 500 series barb adaptor (part # FTLL004-1) from Value Plastics, Inc. The catheter was gently inserted into the genital pore as far as possible, and then was slowly removed while applying suction with the syringe. The distance the tubing could be inserted depended on the size and reproductive status of the fish. In general, in smaller fish (<800 mm FL) the tubing was inserted approximately 4-8 cm, while in larger fish the tubing could be inserted farther (8-12 cm or more in some cases). In females that were reproductively active the catheter was inserted the same distance based on size described above, but because of the enlarged size of the ovaries it could be inserted to a greater distance to obtain a larger oocyte sample if desired. Milt samples were also obtained via catheterization for several males that did not express milt following the same procedure outlined above. All samples obtained from the catheter were placed in 20 ml scintillation vials containing

5 ml of 10% phosphate buffered formalin (PBF). The catheter was rinsed with deionized water between each use.

A subsample of fish that did not express milt and that yielded no sample from catheterization were sacrificed for validation of the sex determination. These sacrificed fish were initially sexed in the field based on the appearance of the urogenital area. In the lab, these same fish had their urogenital area wiped clean to remove any expelled reproductive material and waste by a colleague not involved with the sex determination project. Each fish was then re-sexed without *a priori* knowledge of the fish's identification or its initial sex as determined in the field. The fish's actual sex was then determined by direct visual inspection of the gonads.

### **Maturation Staging of Females using Urogenital Catheterization**

To investigate the maturation status of female fish that were catheterized, the oocyte samples were viewed under a dissection microscope at 10-50X depending on the size of the oocytes. A Motic<sup>®</sup> Imaging System was used to measure the diameter of 50 oocytes or as many as possible when there were less than 50 measurable oocytes extracted via catheterization. All hydrated oocytes were measured. The measured oocytes were classified as primary growth oocytes (up to late perinucleolus stage), early development oocytes (stages between late perinucleolus stage and up to cortical alveolus stage), late development oocytes (lipid granule stages), and hydrated oocytes (fully hydrated oocytes) based on their size and general appearance (Grau et al. 1996; Micale et al. 1999; Poortenaar et al. 2001; and Harris et al. 2004, 2007). Degraded oocytes were not measured, but their presence was noted. Based on the most advanced type of oocytes present in the catheter samples, an individual female was classified as immature/resting (primary growth oocytes), early developing (early developing oocytes), late developing (late developing oocytes), ripe (hydrated oocytes or late developing and degraded

oocytes), or spent (early developing and degraded oocytes, but no late developing oocytes) (Grau et al. 1996; Micale et al. 1999; Poortenaar et al. 2001; and Harris et al. 2004, 2007) (Table 2-2). The size frequencies of oocytes in these stages were plotted and compared to ranges given in Grau et al. 1996, Micale et al. 1999, and Harris et al. 2007. No differentiation could be made between immature and resting fish, as this differentiation is based mainly on smaller oocyte stages that are not easily extracted with catheters and on differences in the thickness of the ovarian wall and the presence of muscles bundles in the ovarian lamellae (Grau et al. 1996; Mackie 2000; Harris et al. 2004, 2007). Numbers of fish classified in each maturation stage for each 100 mm FL size class were calculated on a monthly basis, which was used to determine the size of fish and time of year that catheterization provided the most detailed information regarding reproductive stage.

### **Sex Ratio Determination**

Sex ratios of greater amberjack in the Gulf of Mexico were determined using published literature or data sources, as well as applying non-lethal sexing of fish collected in field-based sampling as an alternative method. Overall sex ratio estimates have been given in several prior studies focusing on age, growth, and reproduction of greater amberjack in the Gulf of Mexico and the US South Atlantic. The dataset used in an age, growth, and reproduction study of Gulf of Mexico amberjack by Murie and Parkyn (2008), which contained sex information on over 1600 individuals, was analyzed for estimates of overall sex ratio, as well as sex ratios based on several size classes. Sex ratios of fish < 700 mm fork length (FL) were analyzed to estimate the sex ratios of fish below the recreational size limit. The current (2011) recreational size limit is 762 mm FL, however, from 1990-2008 the recreational size limit was 711 mm FL (28 in), and 700 mm FL therefore represents the nearest 100 mm FL size class to this size regulation. Sex ratios of fish  $\geq$  700 mm FL were analyzed to estimate the sex ratios of fish vulnerable to recreational

fishing and later in their life ( $\geq 914$  mm FL) to commercial fishing. The sex ratios of fish  $\geq 1000$  mm FL were analyzed as it has been noted in a number of previous studies that greater amberjack over a meter in length are predominantly females (Beasley 1993; Thompson et al. 1999; Harris et al. 2007). Annual sex ratio estimates from the Murie and Parkyn (2008) dataset were restricted to 2002-2008, as yearly sample sizes prior to 2002 were low ( $<50$  sexed fish per year). These annual sex ratios were calculated to give an estimate of the range of the overall observed sex ratios, in addition to an overall sex ratio for all years combined.

As an alternative method of calculating sex ratios, data from greater amberjack that were non-lethally sexed in conjunction with an ongoing tag-and-release study in the Gulf of Mexico and off the Florida Keys (D. Murie and D. Parkyn, University of Florida, unpublished data) were analyzed. Sex ratios were calculated in the same manner as for the Murie and Parkyn (2008) dataset for consistency, and separate estimates were derived for fish sampled in the Gulf of Mexico and from US South Atlantic waters off the Florida Keys.

## **Results**

### **Sex Differentiation using Urogenital Pores**

Urogenital pores of both male and female greater amberjack were surrounded by white, papilla-like folds of tissue (Figures 2-2 and 2-3). In addition, both males and females were found to have separate urinary and genital pores. However, the positions of these pores in relation to one another were different. In males, the genital pore lies along the midline with the urinary pore located directly posterior to it. The two pores are separated from one another by a thin (generally  $\leq 1$  mm), flesh-colored septum (Figure 2-2). The septum dividing the two pores extended over the urinary pore and on insertion of a probe into the urinary pore it generally covers the genital pore and vice versa, making it difficult to observe both pores at one time. In females, both the genital and urinary pores were observed to either both lie along the midline or to have one pore lie along

the midline and one pore to be positioned slightly off-center. The two pores were separated by a greater distance than in males, and in most cases the tissue between the pores was at least partially white in color. In some cases the white, papilla-like folds of tissue that surround the urogenital pores extended between the two pores in females (Figure 2-3). The greater separation of the pores in females allowed for easier viewing of both pores simultaneously, even upon insertion of a probe, compared to males. Observation of live mature females in spawning condition revealed that their genital pore was much larger than that of males and was often crescent-shaped (Figure 2-4).

### **Field-based Sex Identification using Urogenital Pores and Accuracy of Sex Determination**

A total of 379 greater amberjack were sexed in the field via characters associated with the urogenital pores (204 males and 175 females). Of these, verification of sex was obtained for 194 individuals (95 males and 99 females). Verification was obtained mainly via expression of milt for males and via catheterization for females (Figures 2-5 and 2-6). Males <800 mm FL had their sex verified primarily by catheterization (Figure 2-6). Only 4 individuals (2% of the verifications) had their sex verified through dissection (Figure 2-5).

In total, 193 fish were sexed correctly yielding an overall accuracy of 99.5%. All males ( $n = 95$ ) were sexed correctly in the field, and females ( $n = 99$ ) were sexed correctly 99.0% of the time in the field (Figure 2-7). The one individual that was incorrectly sexed in the field was a female that was sacrificed, and she was correctly sexed in the lab using characters associated with the urogenital pores prior to direct observation of her gonads via dissection. Both male and female greater amberjack of all sizes were accurately sexed, except for the one female that was 636 mm FL (Figure 2-7).

## **Maturation Staging of Females using Urogenital Catheterization**

All stages of maturation were observed in females catheterized over a sampling time frame of March to November, including females with oocytes classified as immature or resting (Figure 2-8A), in stages of early development (Figure 2-8B), in late stages of development or ripe and spawning (Figure 2-8C), and spent (Figure 2-8D). Of the 97 catheter samples of oocytes obtained, 92 could be staged (Table 2-3) according to the criteria outlined in Table 2-2. Females catheterized ranged in size from 534 mm FL to 1412 mm FL (Table 2-4) and maturity stages of early development and late development could be differentiated in females as small as 800 mm FL (Table 2-4). In addition, a number females >800 mm FL collected during the peak of the spawning season (March-May) could be identified as actively spawning (ripe) based on the presence of hydrated oocytes or the co-occurrence of lipid granule stage oocytes and degraded oocytes from a prior spawning event (Tables 2-3 and 2-4).

The mean diameter of measured oocytes showed distinct separation in the sizes of each category of oocyte used to determine maturation status of catheterized females (Figure 2-9). This size separation in oocyte categories indicated accurate classification in determining the maturation status of females.

Catheter samples from five fish did not contain visible oocytes when examined at magnifications up to 50x. However, the tissue obtained from these five fish did not resemble milt in color or texture, but did resemble tissue surrounding oocytes from other samples both in color and texture. Also, at higher magnification (up to 100x), some structures that loosely resembled oocytes were visible. These samples were all relatively small and likely came from immature or resting females.

## **Sex Ratio Determination**

Overall sex ratio estimates for greater amberjack in the Gulf of Mexico indicated that it was near 1:1 (non-lethal sexing) or had a moderate female skew (Murie and Parkyn 2008 dataset) (Table 2-5). Yearly sex ratio estimates from 2002-2008 from the Murie and Parkyn (2008) dataset had a mean value of 0.55:1 (m:f) but showed variation in the degree of female-skewing for the various years (Figure 2-10). Beasley (1993) and Thompson et al. (1999, which include Beasley's data) has previously reported an overall moderately female-skewed sex ratio for greater amberjack off Louisiana, with fish >1000 mm FL showing a marked female-skewed sex ratio (Table 2-5).

An overall male to female sex ratio of 1.07:1 was obtained for fish from the Florida Keys (US South Atlantic) via non-lethal sexing (Table 2-5). Previous sex ratio estimates for greater amberjack from the US South Atlantic stock indicated a near 1:1 or moderately male-skewed (1.5:1) sex ratio (Burch 1979, Harris et al. 2008) (Table 2-5).

Estimates of sex ratios for fish <700 mm FL and for those  $\geq$ 700 mm FL from the Gulf of Mexico were relatively similar to their corresponding overall sex ratios (Table 2-5). However, sex ratios based on the dataset of Murie and Parkyn (2008) indicated a female-skewed sex ratio for all sizes of fish.

Sex ratio estimates for fish >1 m FL were female skewed in both the non-lethal sexing of fish in the Gulf of Mexico and the Florida Keys, as well as in the dataset of Murie and Parkyn (2008) for the Gulf of Mexico (Table 2-5). Previous sex ratios estimated for fish > 1 m FL have also shown female skewing (Beasley 1993; Thompson et al. 1999; Harris et al. 2007). Overall, the average sex ratio for fish > 1 m FL in the Gulf of Mexico was  $0.43 \pm 0.02$  ( $\pm$  SE).

## Discussion

The use of urogenital pore characteristics to non-lethally sex greater amberjack in the field yielded accuracies greater than or comparable to (99 and 100% for females and males, respectively) a number of previous studies using similar methods on a variety of species summarized in Table 2-1. The accuracies obtained in this study were also comparable to or greater than the accuracies obtained in studies using a number of other possible non-lethal sexing techniques on different species, including steroid, hormone, and protein levels, surgical biopsy, endoscopy, and ultrasound imaging (Table 2-1). The method of sex determination used for greater amberjack in this study was adapted from these existing methods used on other species and it is likely that the general approach could therefore be applied to other species found to be sexually dimorphic with respect to their urogenital pores. For example, this method could easily be adapted to other *Seriola* species, both those found in the Gulf and elsewhere in the world. One relatively large female almaco jack *S. rivoliana* that was retained during sampling for this study had the same urogenital features exhibited by greater amberjack.

The single, small female that was incorrectly sexed in the field was sampled during one of the first applications of the urogenital pore method on live individuals, and she was later successfully identified in the lab as a female prior to dissection. Other than this one female, the method of sexing greater amberjack using urogenital characteristics was accurate regardless of sex or size of fish. Perhaps the greatest limitation of applying the method was that fish <500 mm FL had such small urogenital pores that no attempt was made to sex them. Although it was not observed in this study, small urogenital pores may also contribute to incorrectly sexing fish between 500 and 700 mm FL, particularly during the initial training and application of this method. The most common mistake would likely be to misidentify immature females in this size range as being males due to the female's pores being smaller and having less separation than

seen in larger females or mature females within this size range. It may be possible to improve this method for smaller fish by using a magnifying glass, but it may also be the case that the differences observed for larger fish may not have fully developed in smaller individuals. The use of dyes applied to the urogenital area has been used to improve the sexing of some species where male have a common urogenital opening, but for these species the difference is in the number of pores in each sex and not the position of the pores relative to one another (Rakocy and McGinty 1989; Popma and Masser 1999).

The general maturation stage of female greater amberjack was easily obtained by examining oocyte samples extracted using urogenital catheterization. This was not an unexpected outcome, as urogenital catheterization has been used in monitoring egg maturation of this species in prior studies on captive spawning (Kožul et al. 2001; Mylonas et al. 2004). The upper end of the size frequencies of the oocytes measured in this study tended to be slightly larger than those given in Grau et al. 1996, Micale et al. 1999, and Harris et al. 2007, which may have resulted from regional differences in egg diameters or from differences in preparation of the samples. Oocyte diameters in this study were obtained from whole preserved oocytes, while those from the previous studies were obtained from histological sections that may have resulted in some shrinkage. Other than this small discrepancy the egg diameters from this study corresponded well with previous studies. This, along with the distinct separation in the mean diameters of each oocyte type, indicated that the classification of an individual to a particular maturation stage based on the types of oocytes present based on their general appearance was accurate.

Although maturation staging was possible for spawning females, it was not possible to distinguish between immature versus mature but resting females because this distinction is generally reliant on the appearance of tissues other than oocytes, such as the tunic and muscle

bundles, which are not possible to observe using a catheter alone. However, the use of urogenital catheterization could be used to identify potential spawning aggregations of greater amberjack based on the presence of females with oocyte samples that would be classified as hydrated, indicating that that individual was ripe. It also cannot be ruled out that some fish that were assigned a particular maturation stage did not contain more advanced oocytes that were not collected via the catheter, as catheter samples from live fish were not compared to biopsy samples from the gonads of the same individual post-mortem. However, previous studies with different species have shown that catheter samples generally agree with gonad biopsies from the same individuals (Shehadeh et al. 1972; Garcia 1989; Alvarez-Lajonchère et al. 2001; Ferraz et al. 2004). The maturation status of males through obtaining catheter samples was not investigated as it would generally be assumed that if a male were producing milt that it was mature. However, some prior studies have looked at the number or percentage of motile spermatozoa, and the duration of spermatozoa motility, from samples collected via catheterization of captive male greater amberjack prior to induced spawning during their aquaculture (Kožul et al. 2001; Mylonas et al. 2004).

Non-lethal sexing of greater amberjack, as well as other fishes, can have a variety of useful applications. This study was conducted as part of a tag and release study of greater amberjack in the Gulf of Mexico (D. Murie and D. Parkyn, University of Florida, unpublished data) and the non-lethal data on sex obtained from this study are being used to elucidate information on sex-specific migration patterns, growth rates, and mortality rates as tagged fish are recaptured. Additionally, the celerity of this method (< 1 minute per fish in most cases), its simplicity, and the minimal training required, makes it a suitable candidate for obtaining sex data from greater amberjack by on-board observers as well as port samplers, which generally need to use methods

that allow for relatively rapid data collection that do not require a great deal of technical skill (G. Fitzhugh, National Marine Fisheries Service, personal communication).

As with greater amberjack, tagging studies of other species could benefit from sex data that is currently unavailable. Within the Gulf, cobia *Rachycentron canadum* has been a species at the focus of several large tagging studies (Franks et al. 1991; Burns and Neidig 1992; Hendon et al. 2008), but currently there is no means to differentiate their sex externally. Large pelagic species such as marlin (Istiophoridae), swordfish *Xiphias gladius*, and bluefin tuna *Thunnus thynnus* are often tagged with internal archival or pop-off archival tags, but sexing data are generally unavailable (Block et al. 1998, 2005; Bridges et al. 2000; De Metrio et al. 2002). A method using steroid, hormone, and protein levels of muscle biopsy samples has been developed to sex swordfish and bluefin tuna that are landed either gutted or whole, but these fishes are too economically valuable for invasive samples to be taken (Bridges et al. 2000). However, as discussed previously, methods relying on concentrations of these indicators can be costly and may have decreased accuracies in immature individuals and outside of the reproductive season (Martin et al. 1983; Blythe et al. 1994; Martin-Robichaud and Rommens 2001; Moghim et al. 2002; Colombo et al. 2004). If the use of urogenital pore characters could be adapted for large pelagic species, such as these, it could allow for the sexing of at least some of these species in conjunction with tagging studies. In particular, bluefin tuna are often brought aboard a vessel for tagging (Block et al. 1998, 2005; Metrio et al. 2002), which would allow for the use of such a non-lethal sexing method. These are just a few examples of species that may benefit from an attempt to use this non-lethal sexing method. Tagging studies on any fish species would benefit from prior knowledge of sex, which may be useful in determination of sex-specific migration, growth, and mortality. These data are generally unavailable if it is not obtained at the time of

tagging due to the paucity of tag returns with accompanying sex information and the potential for misidentification by those who have recaptured the fish (St-Pierre 1992; D. Murie and D. Parkyn, University of Florida, unpublished data).

The ability to non-lethally sex greater amberjack has also provided an alternative means to estimate sex ratios from fish as small as 534 mm, not just those large enough to land in the fisheries. This can be used in conjunction with sex ratios obtained from more traditional methods, such as port sampling of the landed catch, to provide a range of reasonable values that should be considered in the management of this species. The only previously published overall sex ratio for greater amberjack in the Gulf of Mexico was estimated as 0.4 males to 1 female (Thompson et al. 1999). Sex ratio estimates from the Murie and Parkyn (2008) dataset, both overall and annual, indicated a similar degree of female-skewing in the sex ratio. Sex ratios calculated from the non-lethal sexing data for fish from both the Gulf of Mexico and the US South Atlantic, however, showed minor male-skewing. These were similar to sex ratios obtained by Harris et al. (2007) in the US South Atlantic, as well as to a value of 1:1, which is currently the assumed sex ratio for assessments of the Gulf stock (NMFS 2006). None of the overall sex ratio findings from this study showed the moderately high, male-skewing that was observed by Burch (1979). The overall male-skewing observed by Burch may have arisen, in part, due the time of year and location of his samples. All of the samples collected by Burch were individuals collected from charter boat landing from a single port, with a large number of the samples collected in during three consecutive months. Preliminary analysis of site specific sex ratios from data collected for Gulf of Mexico greater amberjack (Murie and Parkyn 2008; D. Murie and D. Parkyn, University of Florida, unpublished data) has shown that particular geographic locations may have largely skewed (male or female) sex ratios during at least some times during the year

(personal observation). For instance, site-specific sex ratios from fish collected offshore of Apalachicola, FL during March showed male-skewing as large as 11:1 and a male-skew for all locations in that region during that time of approximately 3:1 (G. Smith, University of Florida, unpublished data). Burch's monthly sex ratio estimates show that during the time of year when most of his samples were collected males predominated; while during other months when fewer samples were collected the sex ratios could be near 1:1, male-skewed, or female-skewed.

All previously published sex ratios for greater amberjack > 1 m FL from the Gulf of Mexico and the US South Atlantic, as well as the results from this study, indicated that there was a relatively large female-skew (approximately 70% female) for this size class. This lends support to the notion that the commercial amberjack fishery, with a minimum size limit of 914 mm FL, likely has a higher selectivity for female fish. The female-skewing observed in these larger individuals could arise from faster growth rates that have been observed in female greater amberjack (Harris et al. 2007, Murie and Parkyn 2008), or it could be attributable to some other factor such as greater natural mortality of male greater amberjack.

The female-skewed sex ratio for fish <700 mm FL calculated from the Murie and Parkyn (2008) dataset indicated that if a female-skew in the overall sex ratio does exist for Gulf of Mexico greater amberjack that it may be attributable to some other factors, naturally occurring or otherwise, than size-selective fishing alone since these fish were below the minimum size. However, the results for the same size class from non-lethal sexing data showed no indication of a sex ratio substantially different from the assumed 1:1 sex ratio.

There were potential biases and errors that may have occurred with both methods used to calculate sex ratios in this study. The majority of the Murie and Parkyn (2008) dataset contained samples obtained through port sampling. These port sampled fish may not accurately represent

the true sex ratio of the stock because port sampled fish do not represent the entire catch, but only the portion of the catch that is brought to port. This provides no sex data for any of the discarded fish, and of those fish that are brought to port only a portion are sampled for sex data. In addition, there is little representation from the commercial fishery due to gutting of the fish at sea. There could also be a potential bias in sexes for landed fish due to the size limits imposed on the fishery, since females are in general larger at age and are also predominant in the largest size classes.

The use of non-lethal sexing in conjunction with a tag and release study has provided an alternative method of obtaining sex ratios, which can alleviate some potential biases by allowing samples to be collected for the entire catch. However, there are limitations to this method as well. There is a potential for bias in the overall sex ratio due to highly skewed sex ratios at individual sites skewing the entire dataset. As mentioned above, preliminary analysis of sex ratios for individual sites has indicated that the sex ratio for a particular location can be highly skewed towards one sex or the other (G. Smith, University of Florida, unpublished data). There were no clear spatial or temporal patterns in these site-specific sex ratios that would create a particular bias in sampling a specific location or during a specific time of year, but this possibility cannot be ruled out without further sampling. The differences observed in the estimates of the overall sex ratio and the sex ratio of fish  $\geq 700$  mm FL from the Murie and Parkyn (2008) dataset and the non-lethal sexing data may have arisen in part due to the potential biases discussed above for each method or possibly due to temporal changes in the sex ratios. Differences in the sex ratios for fish  $< 700$  mm FL may have arisen in part due to site-specific or regional skewing of sex ratios. A large number of fish from this size class in the Murie and Parkyn (2008) dataset were obtained from several locations off the coast of Suwannee, FL,

which often showed site-specific female-skewing (G. Smith, University of Florida, unpublished data). This may have led to the female-skewing observed in the sex ratio for this size class from their dataset. Large numbers of fish in this size class from the non-lethal sexing data were obtained from several areas of the Florida coast with different degrees of site-specific skewing in the sex ratios. Both male and female site-specific skewing were observed off Madeira Beach, female skewing was observed off Suwannee, and male skewing was observed off of Apalachicola (G. Smith, University of Florida, unpublished data), resulting in an overall unskewed sex ratio for this size class from non-lethal sexing. Even with the potential shortcomings found in the different methods used to obtain sex data, it is likely that the sex ratios calculated would at least represent a range of likely values that should be considered in the assessment of this stock.

Table 2-1. Summary of non-lethal sexing methods used for a variety of fish species. The type of reproductive information obtained from each study is indicated by an X or the accuracy (%) reported.

Method	Species	Common name	Sex	Maturation status <sup>a</sup>	Reference
Steroid, hormone, and protein levels	<i>Acipenser transmontanus</i>	White sturgeon	85-100	72-100	Webb et al. 2002
	<i>Acipenser transmontanus</i>	White sturgeon	X		Feist et al. 2004
	<i>Epinephelus guttatus</i>	Red hind	X		Heppell and Sullivan 1999
	<i>Epinephelus striatus</i>	Nassau grouper	X		Heppell and Sullivan 1999
	<i>Gadus morhua</i>	Atlantic cod	94-100		Sangalang 1978
	<i>Mycteroperca microlepis</i>	Gag	X	X	Heppell and Sullivan 1999
	<i>Mycteroperca microlepis</i>	Gag	X	X	Heppell and Sullivan 2000
	<i>Oncorhynchus mykiss</i>	Rainbow trout	86-100		Sangalang 1978
	<i>Oncorhynchus mykiss</i>	Rainbow trout		X	Evans et al. 2004
	<i>Oncorhynchus kisutch</i>	Coho salmon	X		Gordon et al. 1984
	<i>Parophrys vetulus</i>	English sole		68-70	Johnson and Casillas 1991
	<i>Salmo salar</i>	Atlantic salmon	X		Le Bail and Breton 1981
	<i>Salmo salar</i>	Atlantic salmon	X		Evans et al. 2004
	<i>Salmo trutta fario</i>	Brown trout	X		Le Bail and Breton 1981
	Palpation	<i>Salvelinus fontinalis</i>	Brook trout	93-100	
<i>Seriola dumerili</i>		Greater amberjack	X		Takemura et al. 1996
<i>Oncorhynchus masou masou</i>		Masu salmon	X		Kano 2005
	<i>Oncorhynchus mykiss</i>	Rainbow trout	X		Kano 2005
	<i>Salvelinus leucomaenis leucomaenis</i>	White-spotted char	96		Kano 2005

<sup>a</sup> Maturation status may include immature vs. mature, pre-spawn vs. post-spawn, and/or reproductive stage.

<sup>b</sup> Fecundity also estimated.

<sup>c</sup> Denotes a study in which a number of methods associated with external urogenital features was investigated.

Table 2-1. Continued.

Method	Species	Common name	Sex	Maturation status	Reference
Surgical biopsy	<i>Acipenser oxyrhincus</i>	Gulf sturgeon	X	X	Parkyn et al. 2006
	<i>desotoi</i>				
	<i>Epiplatys merra</i>	Honeycomb grouper	100	100	Alam and Nakamura 2008
	<i>Morone saxatilis</i>	Striped bass	95		Ritchie 1965
Endoscopy	<i>Acipenser brevirostrum</i>	Shortnose sturgeon		X	Kynard and Kieffer 2002
	<i>Micropterus salmoides</i>	Largemouth bass	97		Driscoll 1969
	<i>Oncorhynchus mykiss</i>	Rainbow trout	X	X	Moccia et al. 1984
	<i>Salvelinus fontinalis</i>	Brook trout	96	96	Swenson et al. 2007
	<i>Salvelinus alpinus</i>	Arctic charr	X	X	Ortenburger et al. 1996
	<i>Scaphirhynchus albus</i>	Pallid sturgeon	X		Wildhaber et al. 2005
	<i>Scaphirhynchus albus</i>	Pallid sturgeon		X	Bryan et al. 2007
	<i>Scaphirhynchus platyrhynchus</i>	Shovelnose sturgeon	75-93		Wildhaber et al. 2005
	<i>Scaphirhynchus platyrhynchus</i>	Shovelnose sturgeon		X	Bryan et al. 2007
Ultrasound Imaging	<i>Oncorhynchus kisutch</i>	Coho salmon	X		Martin et al. 1983
	<i>Acipenser stellatus</i>	Stellate sturgeon	97	X	Moghim et al. 2002
	<i>Clupea harengus pallasii</i>	Pacific herring	X		Bonar et al. 1989
	<i>Epinephelus guttatus</i>	Red hind	X <sup>b</sup>		Whiteman et al. 2005
	<i>Gadus morhua</i>	Atlantic cod	X		Karlsen and Holm 1994
	<i>Hippoglossus hippoglossus</i>	Atlantic halibut		X	Shields et al. 1993
	<i>Hippoglossus hippoglossus</i>	Atlantic halibut	X	X	Martin-Robichaud and Rommens 2001
	<i>hippoglossus</i>				

Table 2-1.Continued.

Method	Species	Common name	Sex	Maturation status	Reference
Ultrasound imaging	<i>Ictalurus furcatus</i> X <i>I. punctatus</i>	Hybrid catfish	80-100	X	Burtle et al. 2003
	<i>Maccullochella peellii peellii</i>	Murray cod	96	X	Newman et al. 2008
	<i>Melanogrammus aeglefinus</i>	Haddock	X	X	Martin-Robichaud and Rommens 2001
	<i>Morone saxatilis</i>	Striped bass	95-99	X	Blythe et al. 1994
	<i>Morone saxatilis</i>	Striped bass	X <sup>b</sup>		Will et al. 2002
	<i>Morone saxatilis</i>	Striped bass	X <sup>b</sup>		Jennings et al. 2005
	<i>Morone saxatilis</i> X <i>M. chrysops</i>	Hybrid striped bass	42-100		Blythe et al. 1994
	<i>Noturus placidus</i>	Neosho madtom	X	X <sup>b</sup>	Bryan et al. 2005
	<i>Oncorhynchus mykiss</i>	Rainbow trout	X	X	Reimers et al. 1987
	<i>Oncorhynchus mykiss</i>	Rainbow trout	X	X	Evans et al. 2004
	<i>Pleuronectes americanus</i>	Winter flounder	X		Martin-Robichaud and Rommens 2001
	<i>Pleuronectes ferruginea</i>	Yellowtail flounder	X		Martin-Robichaud and Rommens 2001
	<i>Salmo salar</i>	Atlantic salmon	X	X	Reimers et al. 1987
	<i>Salmo salar</i>	Atlantic salmon	X		Mattson 1991
	<i>Scaphirhynchus albus</i>	Pallid sturgeon	X		Wildhaber et al. 2005
	<i>Scaphirhynchus platyrhynchus</i>	Shovelnose sturgeon	86		Colombo et al. 2004
	<i>Scaphirhynchus platyrhynchus</i>	Shovelnose sturgeon	59-76		Wildhaber et al. 2005

Table 2-1. Continued.

Method	Species	Common name	Sex	Maturation status	Reference
Ultrasound imaging	<i>Scaphirhynchus platorynchus</i>	Shovelnose sturgeon		X <sup>b</sup>	Bryan et al. 2007
	<i>Verasper moseri</i>	Barfin flounder	X		Matsubara et al. 1999
Urogenital catheterization	Acanthuridae	Surgeonfishes	X		Ross 1984
	<i>Barbodes schwanenfeldi</i>	Tinfoil barb		X	Bailey and Cole 1999
	<i>Centropomus medius</i>	Blackfin snook		X	Alvarez-Lajonchère et al. 2001
	<i>Centropomus parallelus</i>	Fat snook		X	Ferraz et al. 2004
	Chaetodontidae	Butterflyfishes	X		Ross 1984
	<i>Dicentrarchus labrax</i>	European sea bass		X	Asturiano et al. 2003
	<i>Dicentrarchus labrax</i>	European sea bass		X	Mylonas et al. 2003
	<i>Epinephelus marginatus</i>	Dusky grouper		X	Marino et al 2003
	<i>Epinephelus rivulatus</i>	Halfmoon grouper	80-96		Mackie 2000, 2003
	Labridae	Wrasses	X		Ross 1984
	<i>Lates calcarifer</i>	Barramundi		X	Garcia 1989
	<i>Mugil cephalus</i>	Striped mullet		X	Shehadeh et al. 1972
	<i>Scophthalmus maximus</i>	Turbot		X	McEvoy 1983
	<i>Seriola dumerili</i>	Greater amberjack		X	Kožul et al. 2001
<i>Seriola dumerili</i>	Greater amberjack		X	Mylonas et al. 2004	
<i>Tilapia zillii</i>	Redbelly tilapia		X	Coward and Bromage 2001	
External urogenital features	<i>Acipenser</i> spp.	Sturgeon	82		Vecsei et al. 2003
	<i>Ambloplites rupestris</i>	Rock bass	65-98		Noltie 1985
	<i>Esox lucius</i>	Northern pike	91-94		Casselmann 1974
	<i>Hippoglossus stenolepis</i>	Pacific halibut	98		St-Pierre 1992

Table 2-1. Continued.

Method	Species	Common name	Sex	Maturation status	Reference
External urogenital features	<i>Ictalurus punctatus</i>	Channel catfish	99		Norton et al. 1976
	<i>Lepomis macrochirus</i>	Bluegill	100		McComish 1968
	<i>Micropterus salmoides</i>	Largemouth bass	51-92		Parker 1971
	<i>Micropterus salmoides</i>	Largemouth bass	X		Manns and Whiteside 1979
	<i>Micropterus salmoides</i>	Largemouth bass	48-94 <sup>c</sup>		Benz and Jacobs 1986
	<i>Morone chrysops</i>	White bass	X		Sigler 1948
	<i>Oreochromis</i> spp.	Tilapia	X		Popma and Masser 1999
	<i>Sarotherodon</i> spp.	Tilapia	X		Popma and Masser 1999
	<i>Sebastes paucispinis</i>	Boccacio	X		Moser 1967
	<i>Sebastes caurinus</i>	Copper rockfish	X		Murie 1991
	<i>Sebastes maliger</i>	Quillback rockfish	X		Murie 1991
	<i>Tilapia</i> spp.	Tilapia	X		Rakocy and McGinty 1989
	<i>Tilapia</i> spp.	Tilapia	X		Popma and Masser 1999

Table 2-2. Maturation stages of greater amberjack based on general appearance of oocytes from catheter samples following descriptions by Grau et al. (1996), Micale et al. (1999), Poortenaar et al. (2001), and Harris et al. (2004, 2007).

Maturation Stage	Defining oocyte type	Oocyte stages present
Immature/resting	Primary growth	Stages up to late perinucleous stage
Early developing	Early developing	Stages up to cortical alveolus stage
Late developing	Late developing	Stages up to yolk granule
Ripe	Hydrated or late developing and degraded	Stages up to yolk granule and hydrated and/or degraded oocytes
Spent	Early developing and degraded	Stages up to cortical alveolus stage and degraded oocytes, but no yolk granule or hydrated oocytes

Table 2-3. Number of catheterized female greater amberjack classified into each maturation stage described in Table 2-2 by month.

Maturation Stage	Total	March	April	May	June	November
Immature/Resting	21	13	6	0	1	1
Early Developing	5	3	2	0	0	0
Late Developing	23	4	14	5	0	0
Ripe/Running	42	0	25	17	0	0
Spent	1	0	1	0	0	0

Table 2-4. Number of catheterized female greater amberjack classified into each maturation stage described in Table 2-2 by 100-mm FL size class.

Maturation Stage	Total	500	600	700	800	900	1000	1100	1200	1300	1400
Immature/Resting	21	5	4	6	5	1	0	0	0	0	0
Early Developing	5	0	0	0	2	2	1	0	0	0	0
Late Developing	23	0	0	0	0	3	11	5	4	0	0
Ripe/Running	42	0	0	0	2	7	20	5	7	1	1
Spent	1	0	0	1	0	0	0	0	0	0	0

Table 2-5. Overall sex ratios, sex ratios for individuals <700 mm fork length (FL), sex ratios for individuals  $\geq$ 700 mm FL, and sex ratios for individuals  $\geq$ 1000 mm FL for greater amberjack in the Gulf of Mexico and US South Atlantic.

Group	Sex ratio (m:f)	Sample size	Source
Gulf of Mexico			
Overall	0.4:1	351	Thompson et al. 1999
	0.59:1	1526	Murie and Parkyn 2008 dataset (This study)
	1.19:1	258	Non-lethal sexing (This study)
<700 mm FL	0.72:1	293	Murie and Parkyn 2008 dataset (This study)
	1.18:1	48	Non-lethal sexing (This study)
$\geq$ 700 mm FL	0.56:1	1233	Murie and Parkyn 2008 dataset (This study)
	1.19:1	210	Non-lethal sexing (This study)
$\geq$ 1000 mm FL	0.39:1	173	Beasley 1993/Thompson et al. 1999
	0.47:1	202	Murie and Parkyn 2008 dataset (This study)
	0.43:1	10	Non-lethal sexing (This study)
US South Atlantic			
Overall	1.5:1	1202	Burch 1979
	0.9:1	2206	Harris et al. 2007
	1.07:1	176	Non-lethal sexing (This study)
$\geq$ 700 mm FL	1.07:1	176	Non-lethal sexing (This study)
$\geq$ 1000 mm FL	0.52:1	882	Harris et al. 2007
	0.49:1	102	Non-lethal sexing (This study)

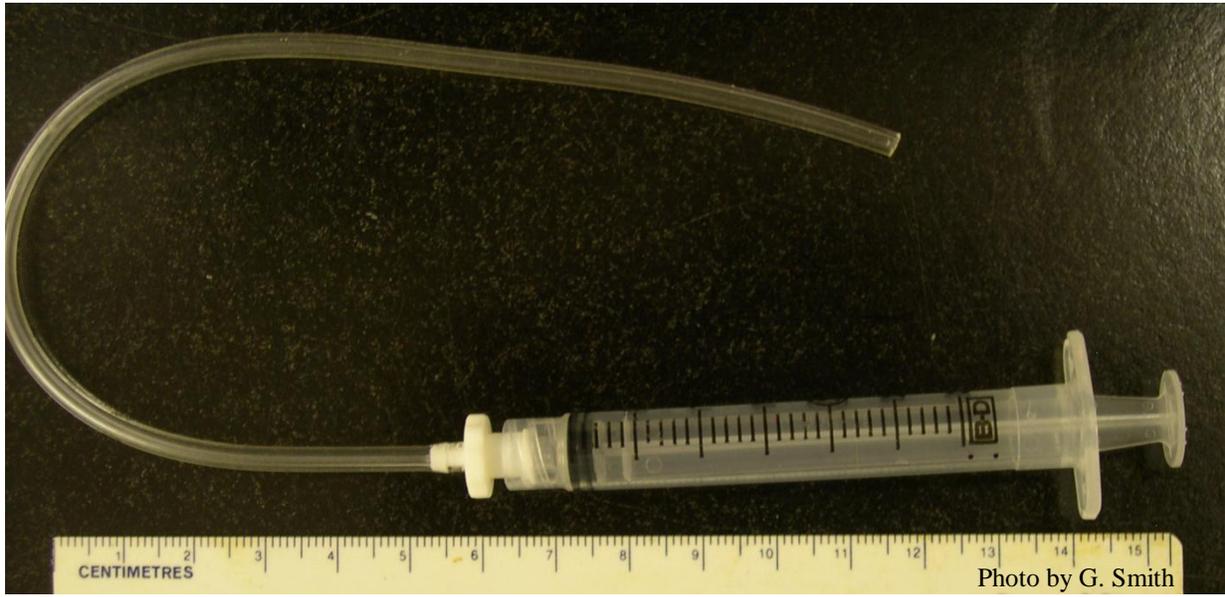


Figure 2-1. Catheter used to obtain milt and oocyte samples from greater amberjack. Photo courtesy of Geoffrey H. Smith Jr.

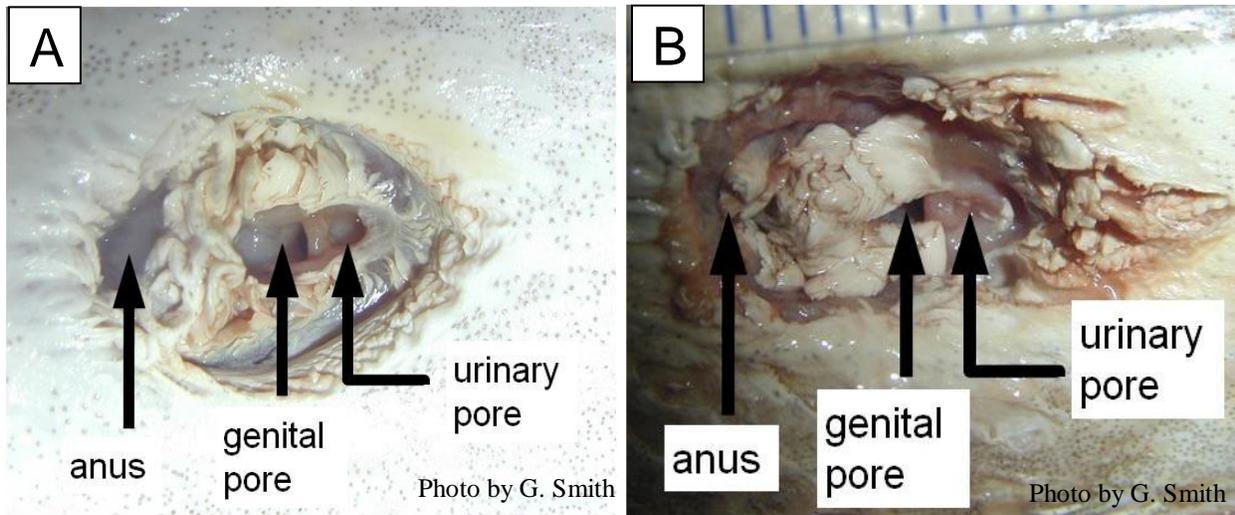


Figure 2-2. Male urogenital region of greater amberjack with anus, genital pore, and urinary pore denoted. The urinary pore is the most posterior structure. A) Genital and urinary pores are both clearly visible in this specimen. B) The urinary pore is partially covered by a septum between the genital and urinary pores in this specimen. Note that the septum is approximately 1 mm in width. Photos courtesy of Geoffrey H. Smith Jr.

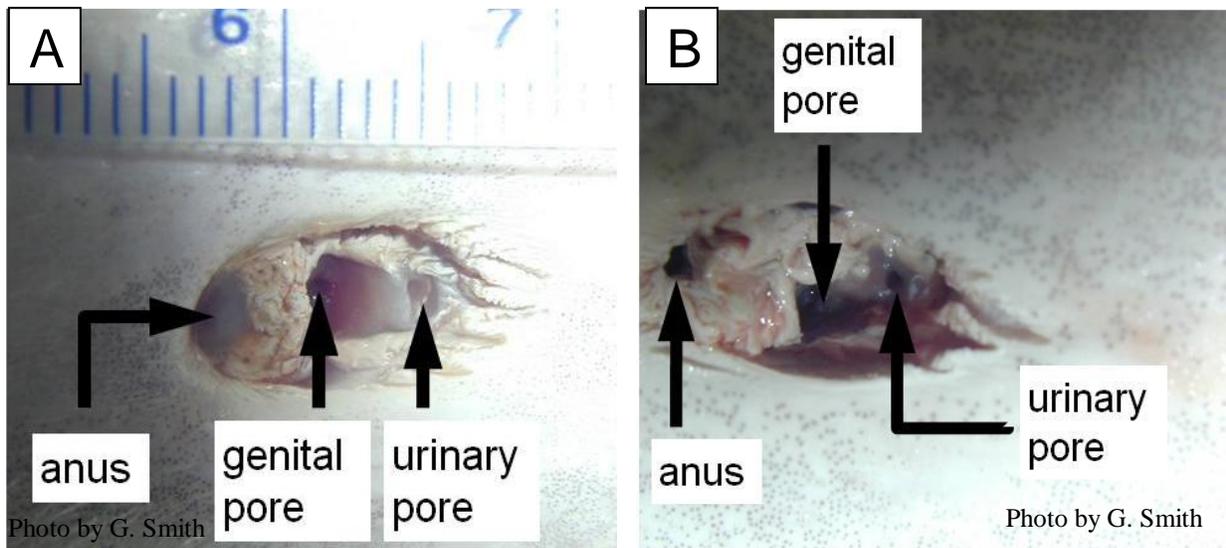


Figure 2-3. Female urogenital region of greater amberjack with anus, genital pore, and urinary pore denoted. The urinary pore is the most posterior structure. A) Septum is greater than 1 mm in width and white in color in this specimen. Some of the papilla-like tissue surrounding the pores is beginning to extend between them. B) Urinary pore is located slightly off the midline in this specimen. Photos courtesy of Geoffrey H. Smith Jr.

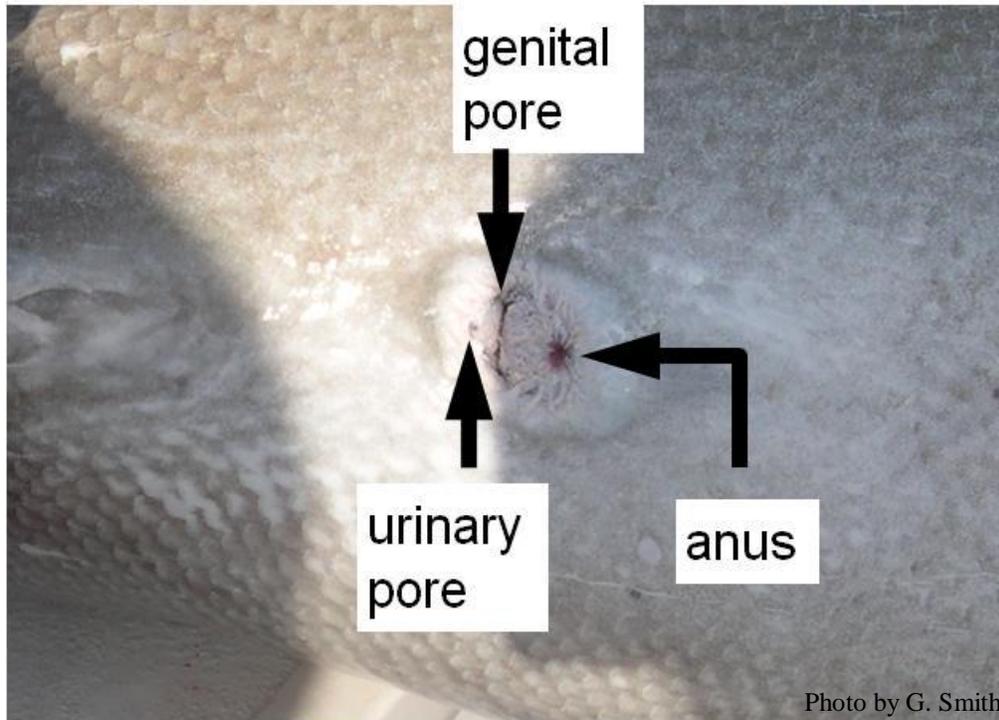


Figure 2-4. Urogenital region of a reproductively active female greater amberjack with the anus, genital pore, and urinary pore denoted. The urinary pore is the most posterior structure. The genital pore is enlarged and crescent shaped, and the papilla-like tissue surrounding the pores has extended between them. Photo courtesy of Geoffrey H. Smith Jr.

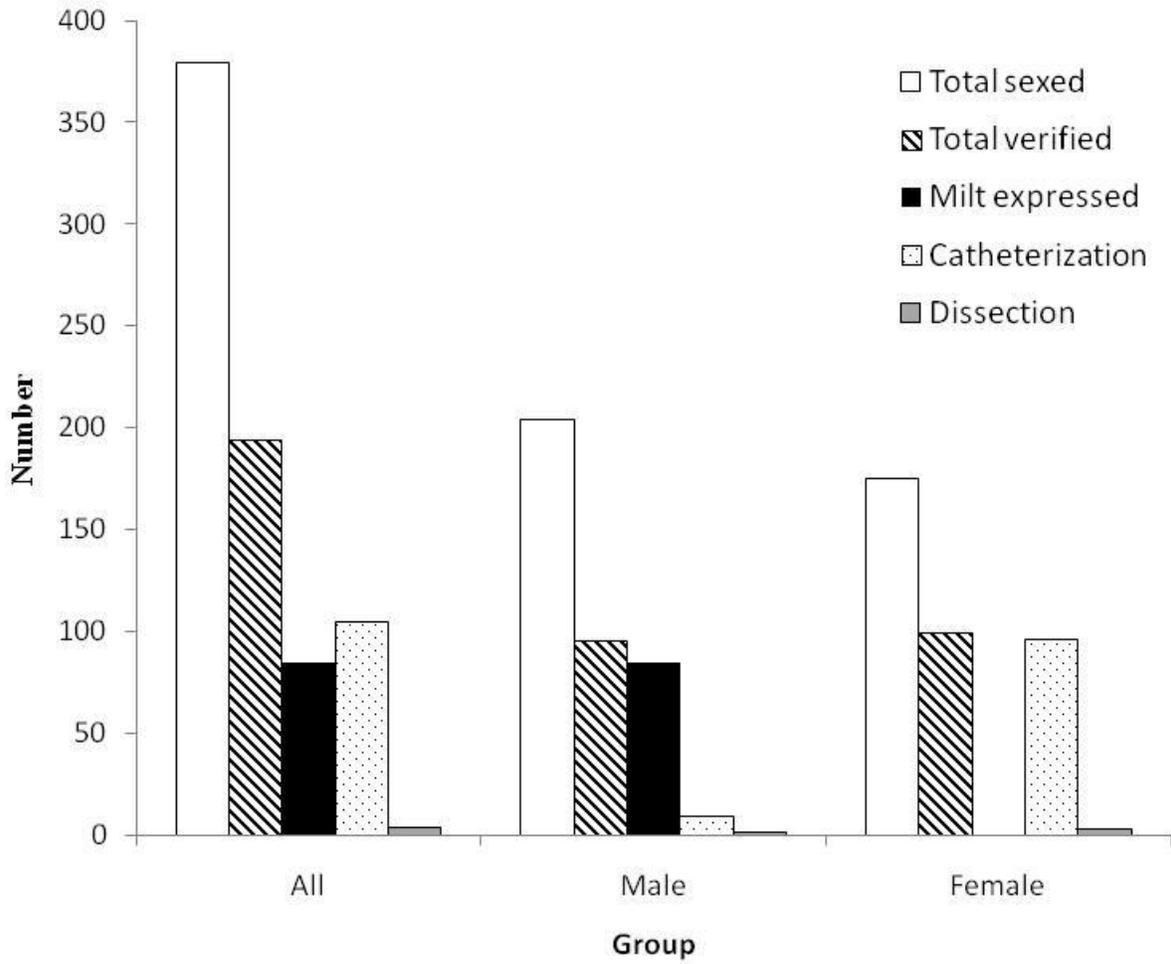


Figure 2-5. Numbers of greater amberjack non-lethally sexed and numbers of greater amberjack that had their sex verified by milt expression, urogenital catheterization, or dissection of sacrificed fish.

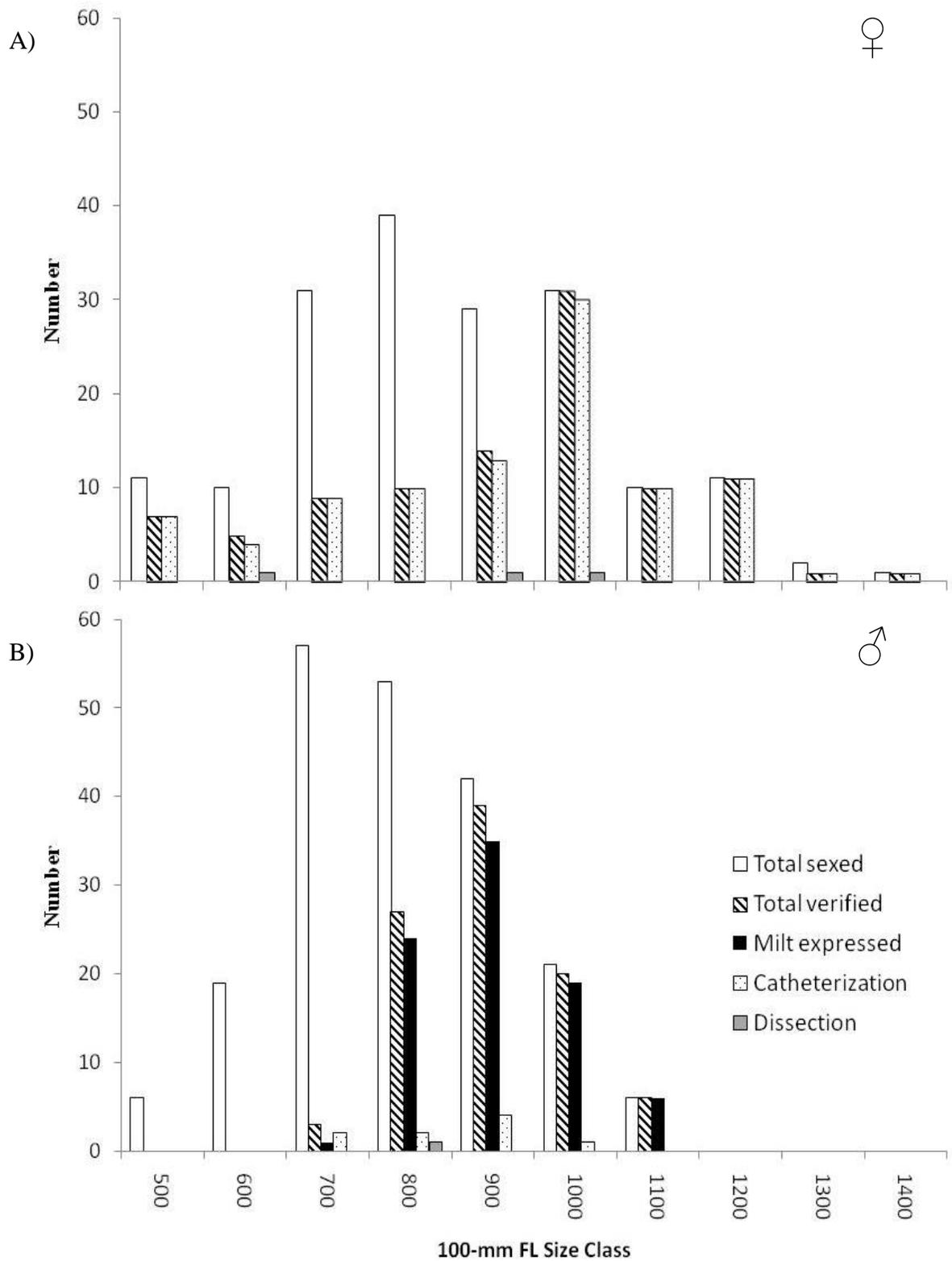


Figure 2-6. Number of female and male greater amberjack non-lethally sexed by size class with sex verified by milt expression, urogenital catheterization, or dissection of sacrificed fish.

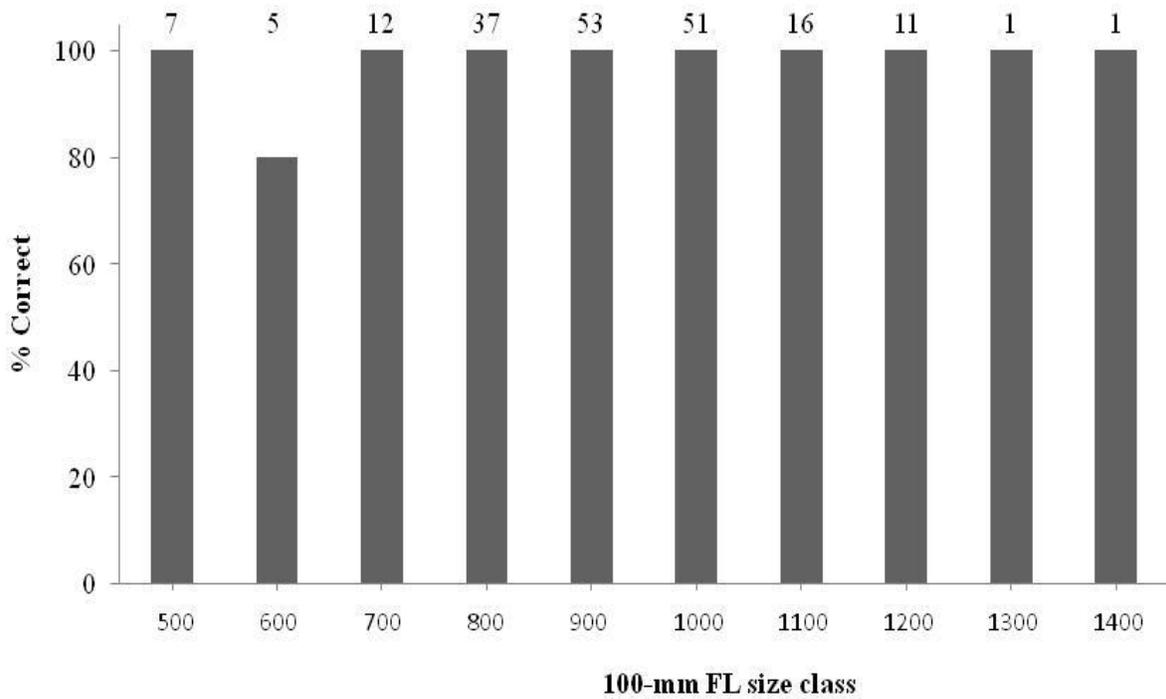


Figure 2-7. Percent accuracy of non-lethal sexing of greater amberjack by size class using features of their urogenital pores. Sample sizes are given above the respective bars for each size class.

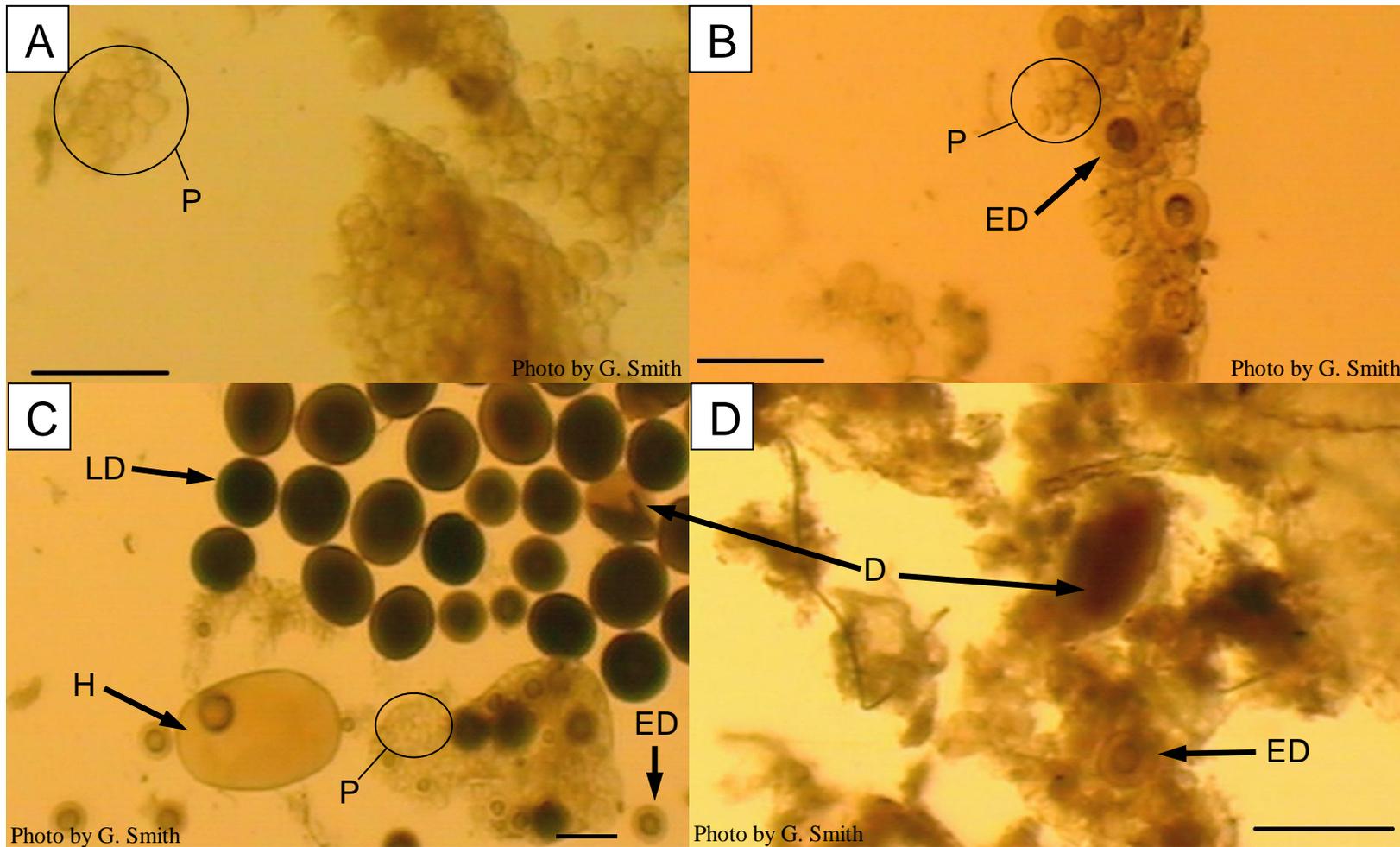


Figure 2-8. Representative images of greater amberjack oocytes at various stages of maturity collected via urogenitalcatheterization: A) female classified as immature or resting (only primary oocytes visible = P); B) female classified as early developing (oocytes up to cortical alveolus stage present = ED); C) female classified as ripe (contains fully hydrated oocytes = H, yolk granule stages are also present = LD); D) female classified as spent (with degraded oocytes = D, but no yolk granule or hydrated oocytes). Scale bar in all images is 0.5 mm. Photos courtesy of Geoffrey H. Smith Jr.

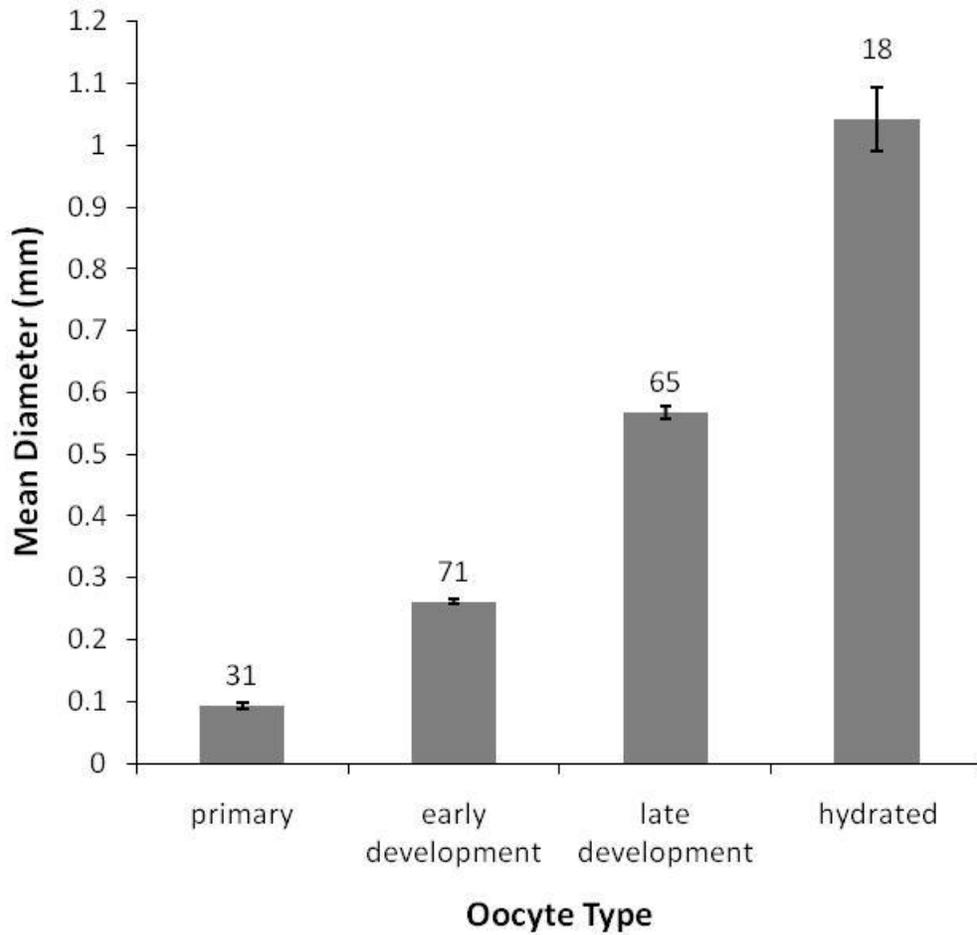


Figure 2-9. Mean oocyte diameter of each oocyte type obtained from urogenital catheter samples of greater amberjack following descriptions given in Table 2-2. Mean oocyte diameters were calculated for each fish for each oocyte type present in the sample and then averaged among fish in which a particular type of oocyte was measured. Error bars represent the standard error of the mean diameter of each oocyte type, with number of fish sampled given above error bars.

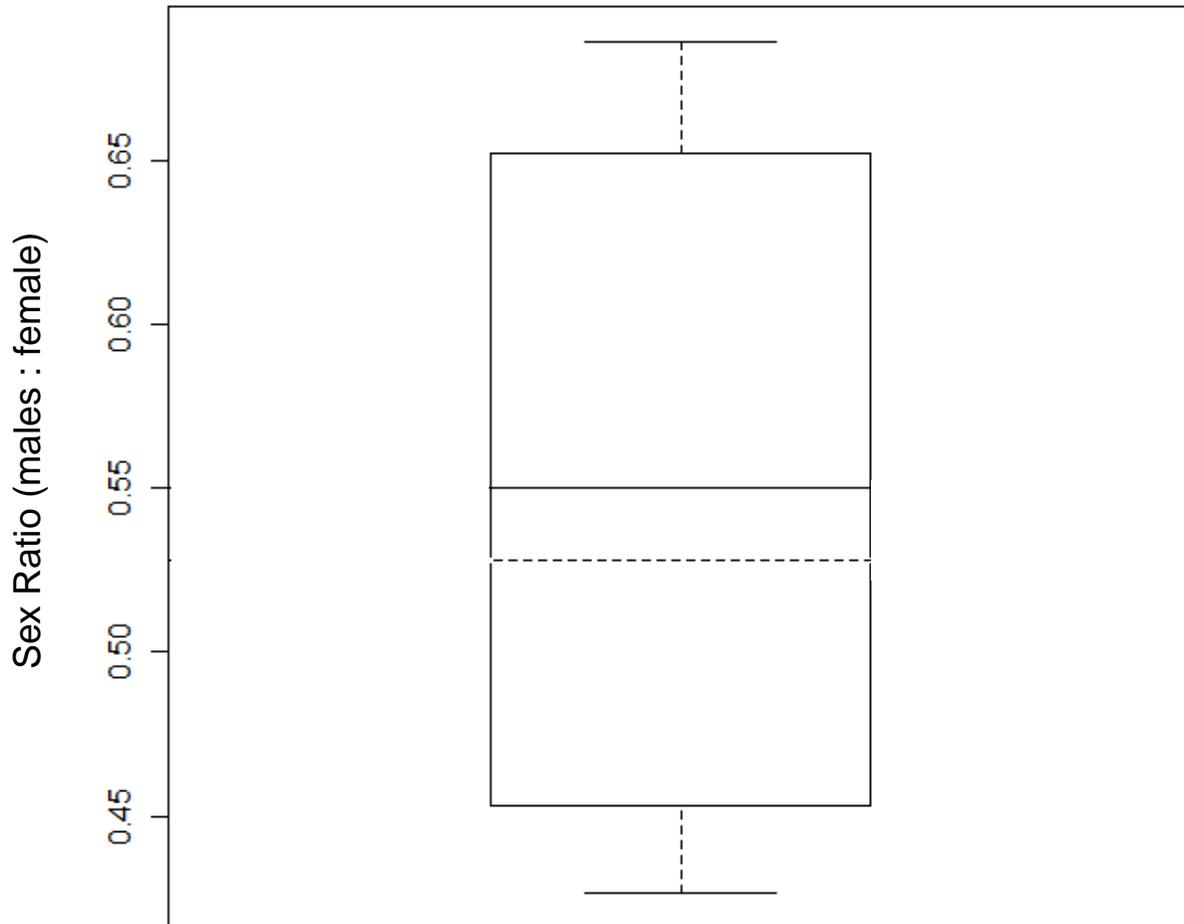


Figure 2-10. Annual male to female sex ratios from the Murie and Parkyn (2008) dataset for 2002-2008. The solid line represents the mean and the dashed line represents the median (2nd quartile). Upper and lower ends of the box represent the 1st and 3rd quartiles, respectively. Whiskers represent the upper and lower range of values observed on an annual basis.

CHAPTER 3  
SEX RATIO EFFECTS ON POPULATION DYNAMICS OF GREATER AMBERJACK

**Overview**

Traditionally, fisheries models tend to focus on growth, reproduction, and survival of a population, with little consideration of behavior, life history strategies, and reproductive patterns. However, there is an increasing realization that proper management requires an understanding of these factors, as well as growth, reproduction, and survival (Alonzo and Mangel 2004, 2005). Gonochoristic, as well as sex-changing, populations tend to have a reduced reproductive capacity as fishing increases due to a decrease in stock biomass and resultant decrease in reproductive individuals (Huntsman and Schaaf 1994). In sex-changing species that undergo size-selective fishing there tends to be a large reduction in the individuals of the larger sex. This leads to an altered sex ratio and a theoretical reduction in reproductive potential either through egg or sperm limitation, which is often predicted to be greater than that seen in gonochoristic species if there is no compensation mechanism (Huntsman and Schaaf 1994; Armsworth 2001; Alonzo and Mangel 2004, 2005; Heppell et al. 2006; Molloy et al. 2007; Alonzo et al. 2008; Brooks et al. 2008).

In protogynous species, including gag grouper *Mycteroperca microlepis*, grasby *Epinephelus cruentatus*, coral trout *Plectropomus leopardus*, and California sheepshead *Semicossyphus pulcher*, models have been developed that incorporate sex ratio and fertility rates in determining recruitment based on the number of fertilized eggs rather than simply the total number of eggs produced, in order to incorporate the potential for sperm limitation (Huntsman and Schaaf 1994; Armsworth 2001; Alonzo and Mangel 2004, 2005; Heppell et al. 2006; Alonzo et al. 2008; Brooks et al. 2008). The ability to estimate biological reference points in protogynous species can be based on female spawning biomass, male spawning biomass, and

total spawning biomass. If the potential for decreased fertilization is weak, female spawning biomass will provide the most accurate estimates. Male spawning biomass will provide the most accurate estimates if the potential is very strong, and total spawning biomass will provide the most accurate estimates if the potential is moderate or unknown (Brooks et al. 2008). In protogynous species, female spawning biomass and total spawning biomass consistently produced relative errors in opposite directions over the range of fertilization rates that were considered probable. This occurs because female spawning biomass never accounts for reduction in fertilization success, while total spawning biomass always does. This theoretically creates a situation where female spawning biomass tends to overestimate the productivity of a stock, while total biomass tends to provide a more conservative effort. This would allow for the use of these two different biomass estimates to bound uncertainty in reference points (Brooks et al. 2008). When all male size classes of a protogynous species are fished, a population will theoretically see greatly reduced recruitment and in many cases the potential for a population crash, but if some male size classes escape fishing, and fertility rates are relatively high, then the potential for a crash is greatly reduced (Alonzo and Mangel 2004; 2005). In populations where a compensation mechanism occurs, such as plasticity in the size at sex change, then these populations may be as resilient as those of a gonochoristic species (Huntsman and Schaaf 1994; Heppell et al. 2006). The greater reduction in reproductive output in protogynous species compared to gonochoristic species may not apply to all levels of fishing mortality as they are not inherently more susceptible to exploitation (Brooks et al. 2008).

Protandry is less common than protogyny in fishes, but there are a number of targeted species such as barramundi *Lates calcarifer*, snook, *Centropomus* spp., and shads *Tenuulosa* spp. that exhibit this form of sex change (Molloy et al. 2007). The potential for egg limitation due to

size-selective fishing exists for these species, but has generally not been incorporated into models. Fu et al. (2001) predicted that a protandrous shrimp *Pandalus* spp. would be more vulnerable to recruitment-overfishing than hypothetical non-sex changing populations if there were no plasticity in size at sex change. Molloy et al. (2007) employed a similar model to those used for protogynous species incorporating sex ratio and fertilization rates to look at the number of fertilized eggs as an indication of recruitment in protandrous species, with white seabream *Diplodus sargus* as a model species. As with protogynous fish, there is, theoretically, a marked reduction in recruitment in size-selective fisheries, in this case due to egg limitation rather than sperm limitation. This effect can be amplified if pre-sex-change individuals, as well as all size classes of post-sex-change individuals, are subjected to fishing pressure because few if any individuals will survive to sex-change to replace the females being removed (Molloy et al. 2007). Again, as with protogynous species, a population may be as resilient as a gonochoristic one if there is some compensatory mechanism to increase reproductive output (Molloy et al. 2007).

The need to understand the effect of males on reproductive output is not limited to protogynous species, as any species in which fishing imposes greater mortality on males than on females may have similar affects (Alonzo et al. 2008), and it is likely that the same would apply to species in which females undergo greater fishing mortality than males. Greater amberjack are not a sex-changing species but the potential exists for differential exploitation of one sex over the other due to size-selective fisheries and evidence of potential sex-ratio skewing (Chapter 2). Greater amberjack are gonochoristic, but show sexual dimorphism in growth with females generally being larger than males at age, as well as dominating the largest size classes. However, this greater growth of females than males at a specific age appears to be less significant in the Gulf of Mexico stock (Murie and Parkyn 2008) compared to the US South Atlantic stock (Harris

et al. 2007), but may still play some role in creating a sex-selective fishery due to size regulations. Minimum size limits make both the recreational and commercial fisheries in the Gulf of Mexico size-selective (762 mm FL (30 in) and 914 mm FL (36 in), respectively, in 2011). In addition, because of an increased minimum size regulation for the commercial fishery, it selects for larger fish, which may consist mostly of females since fish > 1 m FL appear to be comprised of approximately 70% females in both the Gulf of Mexico and US South Atlantic stocks (Chapter 2). Factors other than faster growth rates in females may contribute to this female-skewing, as the differences in growth rates between sexes was less apparent in the Gulf stock, but there was still evidence for female-skewing in these largest size classes. The recreational fishery tends to select fish over 762 mm FL (due to current size regulations), but may have a truncated selectivity of larger fish due to gear limitations, such as line break-offs of larger fish, and travel limitations, such as leaving the larger fish out of reach of anglers on day trips due to their location further offshore, which may possibly lead to more males being harvested. Some preliminary data indicate that site-specific sex ratios of greater amberjack can be highly skewed to one sex or the other (G. Smith, University of Florida, unpublished data), which could be another contributing factor that may lead to sex-selective fisheries in the Gulf stock of greater amberjack.

The overall goal of this study was to examine the effects of sex ratios on the population dynamics of greater amberjack in the Gulf of Mexico. The specific objectives were to apply estimated sex ratios (Chapter 2) to a sex, size- and age-structured population model to 1) determine the effects of male and female-skewed sex ratios on the reproductive potential of the stock, and 2) estimate the effect on parameters of reproductive output from size-selective fishing on females > 1m FL.

## Methods

A two sex, size- and age- structured model was constructed to examine the potential impacts of both male- and female-skewed sex ratios on the reproductive potential of the Gulf of Mexico greater amberjack stock by examining a number of output parameters measuring both male and female contributions to the reproductive potential of the stock. Qualitatively, output parameters that were driven to zero in model simulations were considered to be indicative of a stock collapse. Other possible outcomes for output parameters included a continual decline towards zero, and reaching equilibriums at various levels.

Models were run for 50 years with no fishing mortality for the model to equilibrate, and subsequently 50 years of fishing mortality was applied. Measures of the impact of skewed sex ratios and fishing on reproductive potential included female spawning stock biomass, male spawning stock biomass, total fecundity (egg production), fertility (sperm production), and fertilized egg production. Both spawning potential ratio (SPR) and weighted spawning potential ratio (wSPR) were calculated, as these are measures that are often used to evaluate if recruitment-overfishing is occurring (Mace and Sissenwine 1993; Mace et al. 1996). Spawning potential ratio is defined as the ratio of some measure of productivity on a per recruit basis in the fished to the unfished condition (Goodyear 1990). Weighted spawning potential ratio is defined as ratio of total annual egg production in the fished to the unfished condition (Mace et al. 1996). Greater weight is placed on recruitment in wSPR than SPR, which is mainly influenced by mortality (Mace et al. 1996; NMFS 1996). For this study SPR, was measured as the ratio of fertilized eggs per recruit in the fished condition to the number of fertilized eggs per recruit in the unfished condition, and wSPR was measured as the ratio of the number of fertilized eggs in the fished condition to the number of fertilized eggs in the unfished condition to incorporate both

male and female contributions to the productivity of the stock. The average across 100 simulations was calculated for each of these values.

Models were run with varying sex ratios applied either at the level of recruitment or based on size. Models with sex ratios applied at recruitment were used to examine the effects of “naturally” occurring sex ratios that have arisen from some factor other than fishing. Several different sex ratios were applied in these models. A sex ratio of 1:1 was used as a base case scenario as this is the sex ratio that was assumed in the most recent stock assessment (NMFS 2006). Several of the overall sex ratio estimates from Chapter 2 also fell near 1:1. Male to female sex ratios of 2:3 and 1:2 were modeled based on the findings of the sex ratio analyses of Gulf of Mexico greater amberjack (Chapter 2). Male to female sex ratios of 1:3 and 1:5 were also examined as more extreme cases of sex ratio skewing. Skewing of sex ratios to this degree was not observed in estimates of the overall sex ratio (Chapter 2), however, site-specific sex ratios commonly showed skewing to this degree and in some cases to a greater degree (up to 11:1 in some cases; G. Smith, University of Florida, unpublished data). The reciprocal value of all of these sex ratios (3:2, 2:1, 3:1, and 5:1) were also analyzed to examine the potential effects of male-skewed sex ratios on reproductive potential.

Size-based sex ratios were applied in two different ways. One set of size-based sex ratio models was used to examine the effects of sex ratios that may have arisen from fishing due to possible sex-selectivity in the Gulf of Mexico greater amberjack fisheries, arising from potential geographic skewing of sex ratios and the size-selective nature of these fisheries. Greater amberjack in the Gulf of Mexico reach the minimum recreational size limit over a range of ages due to variability in their growth rates. However, to simplify model calculations, the sex ratios were applied at age three, which is the age at which the von Bertalanffy growth curve (Murie and

Parkyn 2008) predicts Gulf of Mexico greater amberjack to exceed the current recreational size limit (762 mm FL) for both sexes. This is also the age at which the von Bertalanffy growth curve (Murie and Parkyn 2008) predicts that both sexes will exceed 700 mm, which was the cut used to calculate the sex ratios being modeled (Chapter 2). For these models, the sex ratio was assumed to be 1:1 prior to fishing (the first 50 years of the model) and below age three. From age three onward, the sex ratios described above for the previous set of models were applied.

The second set of size-based sex ratio models involved a single 0.43:1 female-skewed sex ratio (i.e., 1 male to 2.3 females), which was applied to fish  $> 1$  m FL. This sex ratio was used to represent the female-skewing (approximately 70% females) observed in the sex ratio analysis of fish  $> 1$  m FL (Chapter 2). As with the previous model, a knife-edge age, rather than a stepped representation of the age at which fish would reach a specific length (1 m FL for this set of models), was used to simplify model calculations. The corresponding age at which both male and females were predicted to be closest to 1 m FL by the von Bertalanffy growth curve for the Gulf stock was age 5 (Murie and Parkyn 2008). The female-skewed sex ratio was thus applied at age 5 and onward. In this set of models, the sex ratio was applied both prior to and after the start of fishing to examine how female-skewing of large individuals could influence productivity in relation to the 1:1 sex ratio, and how fishing could influence any differences that may exist. The sex ratio for fish  $< 1$  m was assumed to be 1:1 for comparison with the sex ratio that was assumed in the most recent stock assessment. For models with sex ratios applied based on size/age, the number of males and females were bounded such that when the sex ratio was applied the number of males and females of a specific age in a specific year did not exceed the corresponding number of males or females 1 yr less in age for the previous year.

Estimates of the current instantaneous fishing mortality rate ( $F$ ) for greater amberjack in the Gulf of Mexico, from the Assessment Workshop of the 2006 Gulf of Mexico greater amberjack stock assessment, covered a range of 0.21-0.60 (NMFS 2006). The model preferred by the Assessment Workshop estimated the current  $F$  to be 0.49 (NMFS 2006). To cover this range of values without exceeding it, a base case scenario  $F$ -value of 0.4 was selected and  $F$ -values 20% in either direction (0.2 and 0.6) were selected as alternative values. The Review Workshop for the 2006 Gulf of Mexico stock assessment preferred a different model provided in an addendum, which estimated the current  $F$  to be 0.86 (NMFS 2006). However, some preliminary model runs indicated probable stock collapses regardless of the sex ratio applied, making any comparisons based on sex ratios impossible.

To incorporate the sex-specific growth rates present in greater amberjack, von Bertalanffy growth parameters for each sex (Table 3-1) were used to determine length-at-age for model scenarios with sex ratios applied at recruitment. The growth model was parameterized as:

$$L_t = L_{\infty} * (1 - e^{-k * (t - t_0)}) \quad (3-1)$$

where  $L_t$  is FL (mm) at time  $t$ ,  $L_{\infty}$  is the asymptotic FL (mm),  $k$  is the growth coefficient, and  $t_0$  is the hypothetical age at zero length. In models with sex ratios applied at recruitment, separate growth and mortality schedules were applied to each sex, as the sex ratio was applied before any mortality has been modeled. For models varying sex ratio based on size, separate growth and mortality schedules for each sex could not be applied at the same time that a sex ratio was being applied at a specific age/size. Doing so would create a circular loop in the model calculations. To apply the various sex ratio scenarios, a single von Bertalanffy growth curve was used for both sex (Table 3-1). This most likely resulted in some loss in the ability to model differences in mortality rates for males and females arising from different growth rates for each

sex. However, the sex ratios applied in the first set of size-based models were themselves intended to represent sex-selective mortality arising from size-selective fisheries or sex-selectivity arising from other factors, such as geographically-specific sex-skewing. The difference in growth between males and females for the Gulf stock was also relatively small, making the loss of information from using a single growth curve minimal.

The weight-at-length relationship for males and females was described by:

$$WT = a * FL^b \quad (3-2)$$

where WT is the whole weight (kg), and a and b are constants in the length-weight relationship (Table 3-1). The weight-at-length relationships for males and females were pooled because Murie and Parkyn (2008) found no significant difference between the sexes.

The number of fish at age  $a$  and time  $t$  in the unfished condition (for each sex) was determined as:

$$N_{a,t} = N_{a-1,t-1} * e^{-M} \quad (3-3)$$

where:  $N_{a,t}$  is the number of fish at age  $a$  and time  $t$ ,  $N_{a-1, t-1}$  is the number of fish of the previous age in the previous year, and  $M$  = instantaneous natural mortality rate. A value of  $M$  equal to 0.25 was used based on the baseline value used in the 2006 Gulf of Mexico Stock Assessment (NMFS 2006) (Table 3-1).

The number of fish at age  $a$  and time  $t$  in the fished condition (for each sex) was calculated as:

$$N_{a,t} = N_{a-1,t-1} * e^{-M} * [(1 - U * HL_{t-1} * (P_{cl} + (1 - P_{cl}) * D)) * (1 - U * LL_{t-1} * (P_{cl} + (1 - P_{cl}) * D)) * (1 - U * HB_{t-1} * (P_{rl} + (1 - P_{rl}) * D)) * (1 - U * CBPB_{t-1} * (P_{rl} + (1 - P_{rl}) * D))] \quad (3-4)$$

where:  $U$  is the annual exploitation rate ( $U = (F * (1 - e^{-Z})) / Z$ ), where  $F$  = instantaneous fishing mortality rate and  $Z$  = instantaneous total mortality rate, and  $Z = F + M$ ; HL, LL, HB, and CBPB are the gear selectivities for commercial handline gear, commercial longline gear, recreational headboat fishery, and the combined charter and private boat recreational fishery, respectively (Table 3-2);  $D$  = discard mortality applied across all ages and fisheries (Table 3-1);  $P_{cl}$  and  $P_{rl}$  = the proportion of fish at age that are of legal size for the commercial and recreational fisheries, respectively ( $P_{cl/rl} = 1 / (1 + e^{-(L_t - MSL) / \sigma})$ ), where MSL is the commercial or recreational minimum size limit and  $\sigma$  is a parameter that incorporates the variability in length-at-age. The value of  $\sigma$  is often set at 10% of a particular length of interest, in this case MSL (Coggins et al. 2007; Pine et al. 2008; Tetzlaff et al. 2011). The ratios of the difference in the upper and lower estimates of length-at-age estimates and mean length-at-age estimates for Gulf of Mexico greater amberjack, which were calculated from mean values and standard errors of von Bertalanffy growth parameters from Murie and Parkyn (2008), ranged from approximately 0.05 to 0.13. Based on this information,  $\sigma$  was set at 10% of MSL. These latter values were calculated for each sex to incorporate the difference in size-at-age between males and females.

The number of mature males and females ( $N_{mat}$ ) for each year was calculated as:  $N_{mat} = \sum_a N_{a,t} * P_{mat}$ , where:  $P_{mat}$  is the proportion mature at age  $a$  for each sex based on Table 3-3. Male and female spawning stock biomass (SSB) for each year was calculated as:  $SSB = \sum_a N_{mat} * WT$ , where WT = whole weight (in kg) at age  $a$ .

Batch fecundity at age ( $BF_a$ ) was calculated as:  $BF_a = a_f + (b_f * Age)$ , where  $a_f$  and  $b_f$  are constants in the fecundity-age relationship (Table 3-1). Annual fecundity at age ( $AF_a$ ) was calculated as  $AF_a = n * BF_a$ , where  $n$  = number of spawnings per season (Table 3-1). The total

number of eggs produced each year (EP) was determined by  $EP = \sum_a NF_{a,t} * AF_a$ , where  $NF_{a,t}$  = number of females at age  $a$  for each year.

There is little empirical data on male reproductive parameters in fisheries, which may necessitate the use of theoretical estimates for parameters related to male productivity (Trippel 2003). Sperm production at age ( $SP_a$ ) was predicted to be much greater than egg production, and was therefore estimated to be 1000 times greater than egg production ( $SP_a = 1000 * AF_a$ ) (Alonzo and Mangel 2004; Molloy et al. 2007). Total annual sperm production (SP) was determined by  $SP = \sum_a NM_{a,t} * SP_a$ , where  $NM_{a,t}$  = number of males at age  $a$  for each year.

The proportion of fertilized eggs ( $P_{fegg}$ ), which was a function of the fertilization rate and the proportion of mature males in the spawning stock, was estimated as:  $P_{fegg} = f_{max} * (1 - e^{-\theta * P_{male}})$  (from Heppell et al. 2006), where:  $f_{max}$  is the maximum fertilization rate;  $\theta$  is a fertility parameter that determines the steepness of the curve; and  $P_{male}$  is the proportion of mature males in the spawning stock, calculated as  $P_{male} = N_{mat(male)} / (N_{mat(male)} + N_{mat(female)})$ . The maximum fertilization rate was set at 0.8 based on data from captive spawning experiments with greater amberjack by Jerez et al. (2006); this was the highest average monthly fertilization rate observed in the study. There is currently no empirical data on fertility functions in greater amberjack, thus a theoretical value for  $\theta$  was selected based on Heppell et al. (2006). Heppell et al. (2006) used two values for  $\theta$ , 20 and 80, which represented “low fertility” and “high fertility” functions respectively. The “low fertility” value of 20 for  $\theta$  was chosen because a “high fertility” value would show essentially no changes in fertilization rate with the sex ratios modeled in this study, whereas a “low fertility” should produce at least minor changes in fertilization rate as the sex ratio varies, allowing for investigation of possible sperm limitation in highly female-skewed sex ratios. Total annual production of fertilized eggs (FEP) was calculated as:  $FEP = EP * P_{fegg}$ .

In the 2006 stock assessment (NMFS 2006), recruitment was modeled using a hockey-stick recruitment function (Barrowman and Meyers 2000). However, it was not possible to convert the parameters used in the hockey-stick recruitment function, which were based on spawning stock biomass, to values that would correctly model recruitment based on fertilized egg production used in this study. Recruitment ( $R_t$ ) was therefore estimated using the compensation form of the Beverton and Holt model (Walters and Martell 2004, Catalano et al. 2007) as:

$$R_t = ((\text{recK} / \text{EPR}_0) * E_t) / (1 + ((\text{recK} - 1) / (R_0 * \text{EPR}_0)) * E_t) \quad (3-5)$$

where:  $\text{recK}$  is the recruitment compensation ratio, which represents the ratio of juvenile survival in the unfished condition to juvenile survival in a state where levels have been fished down to near zero; and  $\text{EPR}_0$  is the average unfished lifetime egg production per recruit. This value was calculated by:  $\text{EPR}_0 = \sum_a l_{a_{\text{unfished}}} * \text{AF}_a$ , where  $l_{a_{\text{unfished}}}$  is the unfished survivorship at age  $a$ . The unfished survivorship was calculated as the proportion of fish surviving from the previous year (starting at 1 for the first age modeled) multiplied by the unfished survival rate,  $S$ , where  $S = e^{-M}$ . Because recruitment in this model was being dictated by fertilized egg production in order to incorporate male and female contributions,  $\text{FEPR}_0$  (average unfished lifetime fertilized egg production per recruit) was used in place of  $\text{EPR}_0$ .  $\text{FEPR}_0$  was calculated as  $\sum_a l_{a_{\text{unfished}}} * \text{AF}_a * P_{\text{fegg}}$ .  $E_t$  (same as EP) was the number of eggs produced in year  $t$ , again because recruitment in this model was being dictated by fertilized egg production,  $\text{FE}_t$  (same as FEP) was used in place of  $E_t$ .  $R_0$  was average recruitment in an unfished condition. To incorporate uncertainty in recruitment, a lognormal deviation was applied with a mean of 1 and coefficient of variation of 0.4 (Turner et al. 2000). A  $\text{recK}$  value of 10 was selected based on values from species with similar life histories (Meyers et al. 1999) and from Goodwin et al. (2006) as:

$$\ln(\text{recK}) = 4.69 + 0.32 * \ln(W_\infty) + 0.72 * \ln(T_{\text{mat}}) - 0.25 * \ln(\text{Fec}_{\text{mat}}) \quad (3-6)$$

where:  $W_{\infty}$  is the asymptotic total weight (estimated from Equation 3-2 for the maximum age modeled, age 10),  $T_{\text{mat}}$  is the age where female maturity was 50% (estimated as 3.5 from Table 3-3), and  $\text{Fec}_{\text{mat}}$  is the fecundity at  $T_{\text{mat}}$  (estimated by  $\text{AF}_a$  at  $T_{\text{mat}}$ ).

Effects of sex ratio on reproductive potential were assessed by graphically comparing the mean values of FSSB, MSSB, EP, FEP, SPR, and wSPR from 100 simulations of each model permutation. Sensitivity analysis was performed on several input parameters, including discard mortality ( $D$ ), both fertility parameters ( $f_{\text{max}}$  and  $\theta$ ), recruitment compensation ratio (recK), and unfished recruitment ( $R_0$ ) (Table 3-1). These sensitivity analyses consisted of calculating the percent change in the mean value of 100 simulations for each output parameter in the final year of the model. Percent changes  $\pm 10\%$  from the base case scenarios ( $F = 0.4$  and 1:1 sex ratio for both models of sex ratios applied at recruitment and sex ratios based on size) were considered to be relevant and indicated that a particular parameter disproportionately influenced output parameters requiring accurate estimation for reliable results. The discard mortality values used in the sensitivity analysis (0.0 and 0.4) were based on alternative values compared to  $D$  estimated in the 2006 stock assessment ( $D = 0.2$ ) (NMFS 2006). Values of 0.6 and 1.0 were used for the maximum fertilization rate, as 1.0 is the highest possible value and 0.6 was the average fertilization rate of greater amberjack observed by Jerez et al. (2006) in a captive setting. An alternative value of  $\theta$  was chosen based on a theoretically high fertility value of 80 used by Heppell et al. (2006). The alternative values for unfished recruitment represent the upper and lower bounds of the 95% confidence interval for this parameter (300,000 and 400,000) (Diaz et al. 2005). Alternative values of recK were obtained from fish with similar life histories (Meyers et al. 1999). A value of 5 is among the lowest values reported for fish with similar life histories

and a value of 15 represents an equivalent increase in  $recK$  that is within the upper range of values reported for fish with similar life histories (Meyers et al. 1999).

## Results

### Modeling Effects of Sex Ratios Applied at Recruitment

Male spawning stock biomass (MSSB) was highest, both prior to and after the start of fishing, in scenarios with the most highly male-skewed sex ratios, and decreased with decreasing male-skew in the sex ratios for all values of  $F$  that were modeled (Figure 3-1). The proportion by which MSSB declined in the fished condition in relation to the unfished condition was the same regardless of the sex ratio, however, because of the different starting values the absolute changes in MSSB were much greater in male-skewed than female-skewed scenarios with the assumed 1:1 sex ratio falling in the middle (Figure 3-1). All sex ratios resulted in asymptotic values of MSSB for both an  $F$  of 0.2 and 0.4. In all cases the 1:1 sex ratio fell in between the extremes of the male- and female-skewed scenarios (Figure 3-1). As  $F$  increased the proportion by which MSSB declined in the unfished condition relative to the fished condition also increased, as would be expected, with an  $F$  of 0.6 resulting in values indicative of a stock collapse for the most female-skewed scenarios. It was not clearly evident whether the remaining sex ratio scenarios at this fishing pressure were slowly continuing to decline towards zero or if they had reached an equilibrium at a very low MSSB value (Figure 3-1C).

Female spawning stock biomass (FSSB) showed a similar but reversed pattern to that of MSSB, with the highest values of FSSB occurring in the most female-skewed sex ratios and declining as the sex ratio became more male dominated (Figure 3-2). Again, this occurred for all  $F$ -values that were modeled. Like MSSB, the proportion of decline from the unfished to the fished condition was the same for all the sex ratios for a specific value of  $F$ , but the absolute value of the decline increased with an increase in female-skewing of the sex ratio. Increases in  $F$ -

values resulted in greater declines in the fished FSSB compared to the unfished FSSB. Equilibria were reached by all sex ratio scenarios for an  $F$  of 0.2 and 0.4, with the 1:1 sex ratio falling between the highest values seen in the female-skewed scenarios and the lowest values seen in the male-skewed scenarios (Figure 3-2A and B). An  $F$  of 0.6 resulted in FSSB values indicating a stock collapse in the two most male-skewed scenarios, while it was again difficult to discern whether the remaining sex ratio scenarios were continuing to slowly decline or whether they had reached an equilibrium at very low values (Figure 3-2C).

Sperm production (SP) followed the same trends observed for MSSB, with the greatest values, both prior to and after the onset of fishing, in the model with the greatest male-skewing in the sex ratio, and decreasing with increased female-skewing of the sex ratio (Figure 3-3). An equilibrium was reached for all sex ratio scenarios for an  $F$  of 0.2 and 0.4 with the 1:1 scenario falling between the values for the male- and female-skewed sex ratios (Figure 3-3A and B). As with the previous two output parameters, the proportion of decline from the unfished to the fished condition was the same for all sex ratio scenarios, but the absolute value of the decline increased with increased male-skewing of the sex ratio. The decline from the unfished to fished condition also increased with an increase in the value of  $F$ , with a value of 0.6 resulting in SP values that indicated a stock collapse for the two most female-skewed sex ratios (Figure 3-3C). Again it was not easily discernable as to whether the remaining sex ratio scenarios at this fishing pressure had reached equilibrium at low levels or were slowly continuing to decline.

Both total egg production (EP) and total fertilized egg production (FEP) values mirrored the trends observed in FSSB. The greatest EP and FEP values resulted from the most female-skewed scenarios and decreased with an increase in male-skewing of the sex ratios (Figures 3-4 and 3-5). Once again, the proportion of the change between the unfished and fished

conditions was equivalent for all sex ratios, while the absolute value of the declines increased with increased female-skewing in the sex ratio for both EP and FEP. Increasing values of  $F$  resulted in greater declines in EP and FEP, and at the highest  $F$ -value modeled ( $F = 0.6$ ) both EP and FEP values indicated a likely stock collapse for the most male-skewed scenarios (Figures 3-4C and 3-5C). All sex ratios at  $F$ -values of 0.2 and 0.4 reached stable asymptotic values, while the remaining sex ratios scenarios at an  $F$  of 0.6 appeared to either be continuing to decline or at very low equilibrium values (Figures 3-4 and 3-5). Again, in all cases the 1:1 sex ratio fell between the values of the male- and female-skewed scenarios for all values of  $F$ . There was only minor evidence of sperm limitation for the sex ratios that were modeled. If sperm limitation were occurring it would be detectable in two possible ways: 1) one sex ratio would have a greater FEP but lower EP than a sex ratio with a greater female-skew; or 2) the proportion of FEP/EP within a specific sex ratio scenario would be substantially less than the maximum fertilization rate that was modeled (0.8). There was a slightly decreased value of FEP/EP in comparison to the maximum fertilization rate of 0.8 for the 1:5 female-skewed sex ratio prior to the onset of fishing (Figure 3-6). This deviation from the maximum rate was decreased after the onset of fishing for an  $F$  of 0.2 and was not apparent for higher values of  $F$  after the onset of fishing. There was also a temporary drop in the FEP/EP at the onset of fishing for the 1:5 scenario before the new equilibrium had been reached. The 1:3 sex ratio scenario also showed a small temporary drop in FEP/EP at the onset of fishing prior to equilibrium being reestablished at the maximum value (Figure 3-6).

Weighted spawning potential ratio (wSPR) and spawning potential ratio (SPR) values yielded a logical but unexpected result. Unlike the other output parameter values discussed above, both the proportion of change from the unfished to the fished condition and the absolute

value of the change in wSPR and SPR values was the same regardless of the sex ratio (Figures 3-8 and 3-9). This occurred because these output parameters were themselves proportions measuring the proportional change in some measure of fertilized eggs (FEP or fertilized eggs per recruit) from the unfished to fished condition. Hence their absolute changes reflected the changes in these proportions for different sex ratios. However, since there was no difference in the proportional change of FEP from the unfished to fished condition for different sex ratios, there was no difference in the absolute values of changes from the unfished to fished condition for wSPR or SPR. The various forms of spawning potential ratios were compared to some general reference values, including 0.2 (Mace and Sissenwine 1993), 0.3 (Mace and Sissenwine 1993) and 0.4 (Clark 2002) to determine if recruitment-overfishing was occurring. If the spawning potential ratio was less than the reference value then the stock was considered to be recruitment-overfished.

The final wSPR values were 0.25, 0.09, and 0.03 for  $F$ -values of 0.2, 0.4, and 0.6, respectively (Figure 3-7). These wSPR values indicated recruitment-overfishing for  $F$ -values of 0.4 and 0.6 for all three commonly used SPR reference values, while an  $F$  of 0.2 would produce wSPR values that would indicate recruitment-overfishing for all but the least conservative reference value (0.2). The final SPR values were 0.33, 0.18, and 0.13 for fishing mortality rates of 0.2, 0.4, and 0.6, respectively (Figure 3-8). Again this indicated that  $F$ -values of 0.4 and 0.6 would produce SPR values that were indicative of recruitment overfishing for all three SPR reference values. An  $F$  of 0.2 would produce SPR values indicating recruitment-overfishing at only the most conservative reference point (0.4).

### **Modeling Effects of Sex Ratios Resulting from Fishing**

For all output parameters (MSSB, FSSB, SP, EP, FEP, wSPR, and SPR), the values prior to fishing were equivalent for all sex ratio scenarios due to the assumption of a 1:1 sex ratio prior

to the onset of fishing (Figures 3-9 to 3-16). Following the initiation of fishing in year 51, the greatest values of MSSB occurred with 1:1, moderately male, or moderately female-skewed sex ratios (Figure 3-9), while highly male- or female-skewed scenarios resulted in the lowest values of MSSB. An artifact of applying the sex ratios was apparent at the lowest fishing pressure ( $F = 0.2$ ), which resulted in a peak in MSSB immediately following the start of fishing (Year 51) for highly male-skewed sex ratios (Figure 3-9A). This artifact was not evident at higher fishing pressures (Figure 3-9B and C). At the lowest level of fishing pressure modeled, the more severely male- skewed scenarios (3:1 and 5:1) showed a continuing trend of decreasing MSSB, however it appeared that the rate of decrease was slowing towards an equilibrium value. All other scenarios reached a stable value (Figure 3-9A). As fishing pressure increased, the potential for a stock collapse in male-skewed sex ratios also increased. In the base-case scenario for fishing pressure ( $F = 0.4$ ), a male-skewed sex ratio of 5:1 leads to a complete collapse in MSSB, and a male-skew of 3:1 results in a final value of MSSB that appeared to be following a continuing downward trend towards collapse (Figure 3-9B). The other sex ratio scenarios all reached relatively similar asymptotic values of MSSB. With the greatest fishing pressure modeled ( $F = 0.6$ ), MSSB values for all male-skewed sex ratios, except the 3:2 scenario, indicated a likely stock collapse (Figure 3-9C). The 3:2 sex ratio appeared to either be continuing a downward trend toward stock collapse or to have reached a very low stable level, while all other sex ratio scenarios had reached essentially the same low equilibrium value (Figure 3-9C).

For all values of  $F$ , the greatest FSSB after the onset of fishing occurred in the most female-skewed scenarios and decreased as sex ratios become more male dominated (Figure 3-10). Similar to MSSB, there was an artifact of applying the sex ratios, which resulted in a peak in FSSB for highly female-skewed sex ratios immediately following the start of fishing (Year 51)

(Figure 3-10A). Again, this artifact was not existent at higher fishing pressures (Figure 3-10B and C). At the lowest  $F$ -value modeled ( $F = 0.2$ ), FSSB reached an equilibrium value for all sex ratios (Figure 3-10A). These equilibrium values covered a relatively wide range of FSSB values and decreased with increased male-skewing. The base case sex ratio (1:1) fell inbetween the male- and female-skewed ratios. The two highly male-skewed scenarios (3:1 and 5:1) resulted in a likely stock collapse at an  $F$  of 0.4. All other sex ratios reached equilibrium values following the same order as for an  $F$  of 0.2, but reduced in value and over a smaller range of values (Figure 3-10B). At an  $F$  of 0.6, all male-skewed sex ratios appeared to result in likely stock collapses, while all other sex ratios reached low, but stable values of FSSB (Figure 3-11C).

Sperm production (SP) followed the same pattern as observed in MSSB (Figure 3-11). As with MSSB, there was an artifact present in the male-skewed sex ratios at the lowest fishing pressure that resulted in a peak in SP (Figure 3-11A), but again this artifact was lost as fishing pressure increased (Figure 3-11B and C). Even at the lowest value of  $F$  modeled ( $F = 0.2$ ), the most male-skewed sex ratio (5:1) resulted in a considerable decrease in SP compared to the 1:1 and moderately-skewed (both male and female) sex ratios, and reached a similar equilibrium value to that of the most female-skewed scenario (Figure 3-11A). All sex ratios reached relatively high equilibrium values with moderately male-skewed and 1:1 sex ratios producing the highest values. With an  $F$  of 0.4 the most male-skewed scenario resulted in a value indicative of a stock collapse, and the 3:1 sex ratio produced a continuing downward trend towards collapse (Figure 3-11B). SP was reduced essentially to zero for the most male-skewed sex ratios at an  $F$  of 0.6, while the moderately male-skewed sex ratios appeared to either be continuing a downward trend or a very low equilibrium value (Figure 3-11C). The remaining sex ratios yielded low equilibrium levels of SP that were all approximately the same value.

Total egg production (EP) mirrored the trends observed in FSSB with the highest values after the onset of fishing, for all fishing pressures, occurring in the most female-skewed models and decreasing with an increasing male-skew in the sex ratios (Figure 3-12). The artifact due to applying sex ratios was apparent at an  $F$  of 0.2 in the most female-skewed sex ratios producing a peak in EP (Figure 3-12A). This artifact was not present at higher fishing pressures (Figure 3-12B and C). All sex ratio scenarios for an  $F$ -value of 0.2 resulted in equilibrium levels of EP being reached, with values decreasing with increased male-skewing (Figure 3-12A). For an  $F$  of 0.4, male-skewed sex ratios of 5:1 and 3:1 resulted in no egg production, while more moderately male-skewed scenarios resulted in low equilibrium levels of EP (Figure 3-12B). Moderate male-skewing resulted in EP values that appeared to be continuing a downward trend towards collapse and the most male-skewed scenarios were indicative of a collapse at an  $F$  of 0.6. The remaining sex ratios reached equilibrium levels that decreased with a decreasing female-skew, resulting in the 1:1 scenario having the lowest equilibrium level (Figure 3-12C).

Total fertilized egg production (FEP) followed the trends observed in EP. Again there was a peak in FEP at an  $F$  of 0.2 in the most female-skewed sex ratios due to an artifact of applying the sex ratios (Figure 3-13A). This artifact was lost when  $F$  was increased (Figure 3-13B and C). The highest values of FEP for all values of  $F$  were observed in the models with the highest female-skewing and decreased with an increase in male-skewing of the sex ratios (Figure 3-13). With an  $F$  of 0.2, all sex ratios produced equilibrium values of FEP that decreased as male-skewing increased (Figure 3-13A). The 3:1 and 5:1 male-skewed sex ratios resulted in FEP values indicating a stock collapse at an  $F$  of 0.4, while all other sex ratios resulted in equilibrium levels that followed same pattern seen for an  $F$  of 0.2, but at decreased values (Figure 3-13B). At the highest fishing pressure modeled ( $F = 0.6$ ) the most male-skewed scenario resulted in no

FEP, while moderately male-skewed scenarios appeared to be following a downward trend towards collapse. The female-skewed and 1:1 sex ratios showed the same pattern of equilibrium values observed in the lower fishing pressures, but at decreased values (Figure 3-13C). For this set of models there was no substantial indication of sperm limitation for any of the sex ratios at any of the fishing mortality rates modeled (Figure 3-14). There was a minor, but detectable, drop in FEP/EP in the most female-skewed sex ratio during the first year of fishing prior to an equilibrium being reestablished at the maximum value of 0.8 (Figure 3-14).

For both wSPR and SPR, the greatest values were obtained with the most female-skewed sex ratios and decreased as the sex ratios became more male dominated (Figures 3-15 and 3-16). An artifact of applying the sex ratios was present in the wSPR for an  $F$  of 0.2 for the most female-skewed sex ratios, creating a peak in values in Year 51 when fishing started (Figure 3-15A). This artifact was not present in models with increased fishing pressure (Figure 3-15B and C). This artifact was also not present in SPR values for any of the model scenarios (Figure 3-16). For an  $F$  of 0.2, wSPR values for all male-skewed sex ratios indicated that recruitment-overfishing was likely occurring based on the lowest wSPR reference value of 0.2. The base case sex ratio (1:1) was also considered to be recruitment-overfished based on wSPR reference values of 0.4 and 0.3. All female-skewed scenarios would not be considered to be recruitment-overfished with wSPR reference values of 0.2 and 0.3, and only the two most female-skewed sex ratios would not be recruitment-overfished using a wSPR reference value of 0.4 (Figure 3-15A). For the base-case fishing pressure ( $F = 0.4$ ), all but the most female-skewed sex ratio scenarios indicated that recruitment-overfishing was occurring using the least conservative wSPR reference value (0.2), while at the other two wSPR reference values all sex ratios produced a situation indicative of recruitment-overfishing (Figure 3-15B). The most

male-skewed scenarios completely collapsed, and the moderately male-skewed scenarios exhibited wSPR values indicative of substantial levels of recruitment-overfishing at the least conservative wSPR reference value. All sex ratio scenarios resulted in a designation of being recruitment-overfished for all wSPR reference points at an  $F$  of 0.6, with all male-skewed values indicating a likely stock collapse (Figure 3-15C).

Trends in SPR were similar to those of wSPR, but final values were generally larger than those of wSPR, resulting in fewer scenarios of recruitment-overfishing (Figure 3-16). All female-skewed sex ratios resulted in a designation of not being recruitment-overfished for all three SPR reference values at an  $F$  of 0.2 (Figure 3-17A). The 1:1 scenario would not be considered to be recruitment-overfished at an  $F$  of 0.2 for SPR reference values of 0.2 and 0.3. Moderately male-skewed sex ratios did not indicate recruitment-overfishing for the least conservative SPR reference value (0.2), while the most male-skewed scenarios produced SPR values indicative of recruitment-overfishing for all reference points. In the base case scenario ( $F = 0.4$ ), no sex ratios produced SPR values greater than or equal to the 0.4 SPR reference value, and only the 1:5 female-skewed sex ratio produced an SPR value greater than or equal to the 0.3 SPR reference value (Figure 3-16B). All female-skewed sex ratios produced SPR values that indicated that they were likely not recruitment-overfished using a SPR reference point of 0.2. The 1:1 sex ratio value fell slightly below the 0.2 SPR reference value and all male-skewed scenarios were also considered to be recruitment-overfished for all three SPR reference points. All sex ratio scenarios would result in designations of recruitment-overfishing for the 0.3 and 0.4 SPR reference points for an  $F$  of 0.6, and only the 1:5 female-skewed sex ratio would not be considered recruitment-overfished using a SPR reference point of 0.2 (Figure 3-16C).

### **Modeling Effects of Female-skewed Sex Ratios for Fish $\geq 1$ m FL**

Models based on fish  $>1$  m FL showed that MSSB values for the 1:1 sex ratio were greater at all fishing pressures both prior to and after the onset of fishing compared to the corresponding models with a 0.43:1 female-skewed sex ratio (Figure 3-17A and B). After the onset of fishing, and with an increasing value of  $F$ , the difference in MSSB between the 1:1 and the 0.43:1 scenarios was greatly reduced due to excess males being fished out in the 1:1 scenario. For the 0.43:1 scenario, the MSSB was reduced essentially to zero at an  $F$  of 0.6 (Figure 3-17B), and the corresponding value from the 1:1 sex ratio scenario appeared to be continuing to decrease towards collapse (Figure 3-17A). The MSSB values at  $F$ -values of 0.2 and 0.4 for both the 0.43:1 and 1:1 scenarios reached equilibrium levels, with values of MSSB at  $F = 0.4$  considerably reduced in comparison to  $F = 0.2$  values (Figure 3-17A and B).

Initially, FSSB was greater in the 0.43:1 sex ratio model compared to the 1:1 model due to the greater proportion of females in the largest size classes (Figure 3-18). This trend was maintained for an  $F$  of 0.2, but to a far lesser degree than in the unfished condition. In the base case scenario ( $F = 0.4$ ), FSSB values were nearly identical after the onset of fishing for both sex ratio scenarios, and at an  $F$  of 0.6 the final FSSB values were slightly larger in the 1:1 scenario (Figure 3-18A and B). As with MSSB, the FSSB value for an  $F$  of 0.6 for the 0.43:1 female-skewed scenario was reduced essentially to zero (Figure 3-18B), and the corresponding value for the 1:1 sex ratio scenario was reduced to a very low, but stable level (Figure 3-18A). For lower  $F$ -values, both sex ratio scenarios reached equilibrium levels, however, the values for an  $F$  of 0.4 were greatly reduced in comparison to an  $F$  of 0.2 (Figure 3-18A and B).

Sperm production mirrored the trends seen in MSSB with the 1:1 sex ratio having greater SP values than the 0.43:1 sex ratio for all fishing pressures, both prior to and after the onset of fishing (Figure 3-19A and B). The differences in the values between the two sex ratios were

diminished after the onset of fishing. At  $F$ -values of 0.2 and 0.4 both sex ratio scenarios reached equilibrium values, with the values for an  $F$  of 0.4 being considerably less than the values for an  $F$  of 0.2. The female-skewed sex ratio for an  $F$  of 0.6 showed a continuing downward trend towards collapse, while the 1:1 scenario showed a low, but stable equilibrium level (Figure 3-19A and B).

Both EP and FEP followed the trends observed for FSSB, with the greatest initial values occurring in the female-skewed sex ratio scenarios (Figures 3-20 and 3-21). This trend was maintained throughout the model with an  $F$  of 0.2, but again to a lesser degree than in the unfished condition (Figures 3-20 and 3-21). In the fished condition the EP and FEP values were nearly identical at an  $F$  of 0.4, and with an  $F$  of 0.6 the 1:1 sex ratio scenario had slightly larger values for EP and FEP (Figures 3-20 and 3-21). Again an  $F$  of 0.6 resulted in values that would indicate a stock collapse for the female-skewed sex ratio scenario for both EP and FEP (Figures 3-20B and 3-21B). As with the previous set of size-based models, there was no indication of sperm limitation as FEP/EP remained at the maximum value of 0.8 (Figure 3-22).

For both wSPR and SPR, the final values were greater in the 1:1 sex ratio scenario than the 0.43:1 scenario, but only by a slight margin (Figures 3-23 and 3-24). Final wSPR values for both the 0.43:1 and 1:1 scenarios indicated recruitment-overfishing for wSPR reference values of 0.3 and 0.4, and only at an  $F$  of 0.2 would wSPR values be above the wSPR reference value of 0.2 (Figure 3-23). None of the final SPR values were above the SPR reference of 0.4. The values for both sex ratios with an  $F$  of 0.2 were greater than the 0.2 and 0.3 SPR reference points, while the SPR values for  $F$ -values of 0.4 and 0.6 fell below both of these SPR reference points (Figure 3-24).

## Sensitivity Analyses

An increase in discard mortality ( $D$ ) resulted in a decrease in all output parameters, while a decrease in  $D$  resulted in an increase in all output parameters for both types of models (Tables 3-4 and 3-5). The percent change (22-35%) was considerably greater than 10% for all output parameters for both models with sex ratios applied at recruitment or based on size, except for SPR which showed percent changes that were only slightly greater than 10% (12-14%) (Tables 3-4 and 3-5).

Changes in the maximum fertilization rate,  $f_{max}$ , resulted in a percent change of approximately 26-28% for FEP in the models with sex ratios applied at recruitment (Table 3-4) and a percent change of approximately 17-27% in FEP for the models with sex ratios applied based on size/age (Table 3-5). The percent changes were positive for an increase in  $f_{max}$  and negative for a decrease in  $f_{max}$ . All the remaining output parameters were changed by less than 10% for both types of models, and there was no clear pattern in whether the change was positive or negative based on whether  $f_{max}$  was increased or decreased (Tables 3-4 and 3-5). SPR values were completely unchanged when  $f_{max}$  was changed. The alternate value of  $\theta$ , which defines the steepness of the fertility curve, produced no percent changes greater than 10% in any of the output parameters for either type of model (Tables 3-4 and 3-5). As with  $f_{max}$ , a change in  $\theta$  resulted in no change in SPR (Tables 3-4 and 3-5).

Increasing the virgin recruitment ( $R_o$ ) resulted in a large positive change for most output parameters, with a decrease in  $R_o$  having the opposite effect (Tables 3-4 and 3-5). In contrast to most other parameters, wSPR showed percent changes well below 10% for both types of models and SPR showed no changes (Tables 3-4 and 3-5).

Changes in the previous input parameters resulted in changes in the output parameters that were similar in magnitude for both positive and negative changes of the input parameters.

However, changes in recK input resulted in large, and asymmetrical, differences in the magnitude of change between equivalent positive and negative changes. Decreasing recK resulted in 87-89% negative changes in all output parameters except SPR, which was unchanged, for both types of models (Tables 3-4 and 3-5). Increasing the value of recK resulted in positive percent changes in all output parameters, except SPR that was again unchanged, but these changes (30-41%) were much less than the percent changes (86-90%) for the equivalent decrease in recK (Tables 3-4 and 3-5).

### **Discussion**

Sex ratios skewed toward one sex or the other can have substantial effects on the productivity of a stock regardless of what size/age that skewing begins to occur at. In general, female-skewed scenarios tended to result in higher productivity and greater resilience to exploitation, while male-skewed scenarios often had decreased productivity and resilience to exploitation, with the 1:1 sex ratio scenario generally falling between the two extremes. High degrees of male-skewing often resulted in output parameters indicating a likely stock collapse or output parameters that were following a continuing downward trend towards collapse. Increasing fishing pressure resulted in some of the moderately male-skewed scenarios indicating a likely stock collapse. Often moderately male-skewed scenarios, and at higher fishing pressures the 1:1 and moderately female-skewed scenarios, yielded stable but very low levels for the output parameters. Such low levels could easily be driven to collapse due to some type of perturbation(s) in the system.

For all the sex ratio models in the present study, the driving component of the productivity appeared to be associated with female fish because there was little or no indication of sperm limitation. This was not completely unexpected as instances where sperm limitation does occur are generally associated with protogynous species that can show considerably larger female-

skewing (Heppell et al. 2006). The one instance of minor sperm limitation occurred for the most female-skewed sex ratio modeled (1:5) for a scenario in which the sex ratio was applied at recruitment. However, it is unlikely that the overall sex ratio for greater amberjack would approach this value (Chapter 2), much less the degree of female-skewing that would be required to cause a substantial level of sperm limitation. Other than this one instance of sperm limitation, all plots of FEP/EP showed that all sex ratios yielded a value of 0.8 for this ratio, which is the maximum fertilization rate that could be achieved based on model parameterization. Lower values could be achieved, as evidenced by the one instance of minor sperm limitation; however values of FEP/EP did not decline in most scenarios because the proportion of males in the spawning stock did not decrease by a large enough degree to drop off the asymptote predicted by the fertility function, which is an exponential function.

In modeling sex ratios applied at recruitment, differences in output parameters between different sex ratios were apparent both before and after the onset of fishing. The proportion of the change in a particular output parameter from the unfished to the fished condition was the same for all sex ratios, but the absolute value of these changes were different for each sex ratio. This would tend to indicate that all of the different sex ratios would produce the same outcomes, in terms of being overfished or not, under the same fishing pressure. However, if the sex ratio is different than that which is assumed, then the presumed fishing pressure exerted on females (the sex that drives productivity in this stock) in the stock may actually be higher or lower because of differences in the abundance of females at different sex ratios present prior to fishing. For example, if the sex ratio is assumed to be 1:1, the value assumed in the most recent stock assessment for Gulf of Mexico greater amberjack (NMFS 2006), and fished under this assumption with a quota in place based on the productivity of this sex ratio, but in fact the sex

ratio is male-skewed, then the quota based on the 1:1 sex ratio would increase the actual fishing mortality experienced by females. Such a situation could lead to severe overfishing of the stock.

Some of the commonly used output parameters for models with sex ratios applied at recruitment can be misleading. MSSB and SP were highest for the most male-skewed scenarios, giving the impression that these sex ratios may be the most productive; however, the values of FSSB, EP, and FEP were the highest for the most female-skewed scenarios and lowest for the most male-skewed scenarios. As was stated previously, the female related output parameters were likely the best indicators of the productivity of a particular sex ratio scenario, as there was little or no indication of sperm limitation for all model scenarios (i.e., the productivity was not limited by male abundance). Both wSPR and SPR showed no differences between any of the sex ratios. This resulted from the fact that these output parameters are proportions based on some measure (FEP or fertilized eggs per recruit) of the unfished and fished number of fertilized eggs, which as stated previously were the same for all sex ratios. With no other output parameters for reference, one could potentially think that there were no differences in the number of fertilized eggs produced between the different sex ratios leading to inappropriate management of the stock. The reference values used to determine if wSPR and SPR values are indicative of recruitment-overfishing may be based on life history parameters of a particular species or stock, but there is no set criteria for the selection of the reference value used and few empirical studies examining how to choose a reference value (Mace and Sissenwine 1993). This can lead to a somewhat arbitrary assignment of reference values, and thus spawning potential ratios may be misleading. In the analysis for this study, a range of commonly used reference values (0.2, 0.3, and 0.4) was used, which demonstrated how uncertainty in this value could influence whether a particular scenario would result in recruitment-overfishing. Greater amberjack are a relatively

fast-growing and short-lived species with high fecundity. Such a life history pattern can result in a higher resilience to recruitment-overfishing, in which case a lower reference value such as 0.2 could be used. However, without specific research focused on determining an appropriate reference value the results should be treated with caution and it is advisable to error on the conservative side in stock management.

In models in which sex ratios were applied based on size, differences were not apparent until fishing had begun due to the assumption of a 1:1 sex ratio prior to fishing. Unlike the models with sex ratios applied at recruitment, the most male-skewed sex ratios did not produce the highest MSSB or SP, but, in fact, produced the lowest values for these parameters. This was because in these scenarios a highly male-skewed sex ratio arises from a large reduction in FSSB, which results in an overall decreased population and hence a decrease in the number of males. The most female-skewed sex ratios also produced relatively low values of MSSB and SP due to the necessary decrease in the number of males to produce the female-skewing. This resulted in the 1:1 and moderately skewed sex ratios having the highest values for these two output parameters. Because the most male-skewed sex ratios do not produce the highest values of MSSB and SP, but rather produce the lowest value, they tend not to be as misleading in assessing the productivity of a stock, as was the case for models with sex ratios applied at recruitment. However, there was still no evidence of sperm limitation, which indicated that FSSB, EP, and FEP were the output parameters that would limit productivity. These parameters were the highest in the most female-skewed sex ratios and lowest in the most male-skewed sex ratios. Moderately skewed and the 1:1 sex ratios produced FSSB, EP, and FEP values in between the extremes, whereas these sex ratios produced the highest values of MSSB and SP, resulting in these latter parameters still being somewhat misleading in regards to stock productivity. Female-skewed sex

ratios tended to be more resilient to fishing due to their higher productivity, but this resilience was decreased with increasing fishing pressure.

Spawning potential ratios were more straightforward to interpret for these models as the initial values of FEP and fertilized eggs per recruit were equivalent for all of the sex ratios (the proportions between unfished and fished conditions were different for each sex ratio). In relation to three commonly used reference points (0.2, 0.3, and 0.4) for assessment of recruitment-overfishing, the likelihood of overfishing increased with an increasing male-skew in sex ratio and with increasing fishing pressure. A number of sex-ratio scenarios over the  $F$ -values modeled yielded wSPR and SPR values indicative of recruitment-overfishing, and for the base case scenario ( $F = 0.4$ ) only female-skewed sex ratio scenarios resulted in a designation of not recruitment-overfished regardless of the reference value used. The current  $F$  for the Gulf of Mexico greater amberjack stock is likely 0.4 or higher, and currently this stock is considered to be overfished and undergoing overfishing (NMFS 2006). Therefore, even though this study used different designation criteria for overfished status it is not surprising that most scenarios yielded designations of being overfished.

The model investigating female-skewing in fish over a meter in length demonstrated that initially female-skewing of these large individuals resulted in higher productivity due to the greater values of FSSB, EP, and FEP. This advantage was quickly diminished with the onset of fishing, which resulted in the removal of the excess large females in the female-skewed scenario. At all but the lowest fishing pressure, the 1:1 sex ratio attained a similar or greater level of productivity after the onset of fishing in comparison to the female-skewed scenario.

Overall, female biomass and egg production were likely the driving factors of productivity for the Gulf of Mexico greater amberjack stock. Model scenarios in which the sex ratio was

female-skewed tended to have added resilience to fishing pressure, however, this extra resilience was greatly reduced as fishing pressure increased. The added reproductive output observed in the scenario with female-skewing in the largest size classes was quickly diminished after the onset of fishing. One possibility that could help to maintain the higher productivity resulting from female-skewing, particularly in the largest size classes, would be to impose a slot limit in the fishery, in which fish  $> 1$  m FL or some slightly larger size would have to be released while also maintaining the minimum FL size limit. The potential success of such a measure would in part depend on the survival of fish that had to be released after capture due to the slot limit. The survival of released greater amberjack is currently being studied (D. Murie and D. Parkyn, University of Florida, personal communication). Another potential problem with implementing a slot limit would be determining if it should be applied to both recreational and commercial sectors of the fishery, particularly since under current regulations a large percentage of commercially harvested fish are over a meter in length (commercial minimum size limit = 36 in or 914.4 mm FL). Therefore, for a slot limit to be effective in the commercial fishery the minimum size limit would also have to be reduced.

The sensitivity analysis of several input parameters demonstrated that with these age, size, and sex based models some inputs have substantially more weight on the output parameters than others. This was the case regardless of how the sex ratios were applied.

SPR values were unchanged in the sensitivity analysis of all input parameters, other than discard mortality. This occurred because SPR values were based on an average lifetime per recruit production of some reproductive output (fertilized eggs in this case), which in its simplest form can be reduced to equilibrium values for unfished and fished conditions. These equilibrium values differ on the basis of mortality rates alone in the scenarios modeled. The only other

possibilities that would result in changes in the value of SPR would be if the mortality schedules or fecundity schedules of the fish were altered from the unfished to the fished condition.

Discard mortality ( $D$ ) had a substantial effect on all output parameters. It has also been important in many of the assessment models used for this stock (NMFS 2006), making the determination of its actual value of particular importance. Currently, research is being conducted to examine discard mortality and what factors may influence it (D. Murie and D. Parkyn, University of Florida, personal communication). With such a heavy weight on model outputs, it would be advisable to investigate a range of potential values and potentially error on the side of caution until better estimates of this value have actually been determined. Currently, stock assessment models for the Gulf stock that incorporate discard mortality also conduct sensitivity analyses on this parameter (NMFS 2006).

Both of the fertility parameters,  $f_{max}$  and  $\theta$ , had a very minor impact on the output parameters. The fact that changes in these parameters had little effect on the output parameters supports the notion that male abundance (MSSB) and productivity (SP) were not likely to be limiting factors for the overall productivity of this stock. As mentioned previously, there is little empirical data available on male reproductive parameters, and much of what is known has been derived from captive settings (Trippel 2003). In many cases, models investigating male reproductive potential rely on theoretical values or values derived from similar species (Alonzo and Mangel 2004, 2005; Heppell et al. 2006; Molloy et al. 2007). The values of  $f_{max}$  that were modeled were based on estimates derived from captive individuals that could possibly exceed values seen in the wild, and the values of  $\theta$  that were modeled were based on theoretical values. The mating structure of a species may also have an impact on how male abundance influences population dynamics (Trippel 2003; Alonzo and Mangel 2004, 2005), but there is currently no

data available regarding the mating structure of greater amberjack in the wild. These factors may have influenced the value of the male associated reproductive output parameters (MSSB, SP, and FEP), however, there was such minor evidence of sperm limitation and the fertility parameters had such little influence in the sensitivity analysis that it is likely any differences would be minor. Trippel (2003) outlines a number of possible research areas and methods that could be used to further investigate the reproductive output of males if there were greater concern for sperm limitation in this or other species.

Changes in recruitment parameters can greatly influence the values of the output parameters for these models. This was to be expected as it is generally the case that any variation on a statistical catch-at-age model (forward moving age-based model) is driven by several leading parameters, which generally include one or more recruitment parameters (Walters and Martel 2004). Changes in the value of virgin recruitment ( $R_0$ ) resulted in substantial percent changes for nearly all output parameters. These percent changes were not as large as those for discard mortality; however, the percent change between the original and alternate values of discard was 100% in comparison to a 14% change in virgin recruitment values. The recruitment compensation ratio (recK) was by far the most important of the input parameters investigated in the sensitivity analysis. The percent changes for equivalent increases and decreases in recK were not similar in magnitude as was the case for the other input parameters. It appeared that a decrease in recK had a greater effect on productivity than an equivalent increase in recK. It was more probable that the recK used in this model would be an underestimate of the actual value rather than an over estimate, as most recK values for species with similar life histories were equal to or greater than the selected value and there were only a few that were lower than the selected value (Meyers et al. 1999). However, this study sought to determine if skewing of sex

ratios could influence the population dynamics of a gonochoristic fish species in a similar manner to that seen in sex-changing species and any direct application of this type of model to a stock assessment would benefit from better stock recruitment parameter estimates based on fertilized egg production. Since male abundance and productivity appeared to have little influence on the overall productivity of this stock, this model could be modified to incorporate a recruitment function based on some other measure of female reproductive potential, such as female spawning stock biomass, rather than fertilized egg production. This would allow for the use of the hockey-stick recruitment function employed in the most recent stock assessment for Gulf of Mexico greater amberjack. A modification of this model to incorporate a different recruitment function would then place the weight of  $recK$  on the parameters of the new function, and some accurate measure of virgin recruitment would still be required.

There are some other factors to consider that could improve the model used in this study. For all of the models, the sex ratios and fishing mortality rates were directly applied, but a gradually changing sex ratio and F-value may more accurately simulate changes within the Gulf of Mexico stock. For the models in which sex ratio was applied at a specific size/age, the sex ratios were applied in a knife-edge manner rather than a stepped manner, which would better incorporate variability in size at age. Using a stepped application of sex ratios would likely produce the same general patterns but shifted by a certain degree due to a portion of younger fish experiencing the shifts in sex ratio. It was also assumed that the number of spawnings per year was equivalent for all mature females. This may not be the case, and further research in this area is still needed. This model could, however, be adapted to simulate the effects of varying spawning frequency based on fish size/age.

It is clear that skewing within sex ratios of gonochoristic fish species, whether arising naturally or from sex-selective fisheries, can greatly alter the productivity of a stock. These results were similar to those found for both protandrous (Molloy et al. 2007) and protogynous (Huntsman and Schaaf 1994; Alonzo and Mangel 2004, 2005; Heppell et al. 2006) fish species. In protogynous species, the reduction in productivity generally results from sperm limitation. In this study, there was no evidence of sperm limitation in any of the models. This may arise from several factors. As previously stated, the female-skewed sex ratios that were modeled in this study were considerably less skewed than those of stocks that have demonstrated potential sperm limitation. Another factor that may eliminate the potential for sperm limitation in the greater amberjack stock is the fact that males generally mature earlier than females in this species (Tables 1-1 and 3-3).

It is clear from this study that an incorrect assumption of the sex ratio in a gonochoristic species, such as greater amberjack, could lead to mismanagement of the stock. Assuming that a sex ratio is more male-skewed than it actually is would likely result in underutilization of the stock, while assuming that a sex ratio is more female-skewed than it actually is would likely result in overexploitation of the stock. Currently the Gulf stock is assumed to have a 1:1 sex ratio, and estimates obtained from non-lethal sexing (Chapter 2) appear to support this assumption. However, sex ratio estimates from other data sources, such as port sampling (Chapter 2), point towards an overall female-skewing in the sex ratio. If this is actually the case, the Gulf stock may not be as exploited as current stock assessment models predict. However, if the greater number of females seen in port samples is not due to a female-skew in the sex ratio, but from sex-selectivity of the fisheries (i.e., females are being differentially harvested due to greater growth rates or spatial and/or temporal patterns in sex ratios), then the overall sex ratio

could be being driven towards a male-skew, which would result in an underestimation of the current exploitation by assuming a 1:1 sex ratio. A range of realistic estimates of sex ratios for the Gulf of Mexico stock of greater amberjack should therefore be used in the stock assessment, rather than continuing to simply assume a sex ratio of 1:1. More detailed data on sex ratios may also help to identify potential spatial and temporal trends in sex ratios related to possible sex-specific schooling and/or migration patterns, which in addition to size limits may result in sex-specific exploitation of one sex or the other. Such data could potentially be used to impose geographic or temporal regulations, such as designated closures, either in space or time, aimed at protecting aggregations of female fish, particularly those in the largest size class

Table 3-1. Input parameters for models. Values in parentheses indicate values used in sensitivity analysis.

Parameter	Value	Source
von Bertalanffy growth parameters		
$L_{\infty}$ (mm)		
Male	1196.6	Murie and Parkyn 2008
Female	1279.6	Murie and Parkyn 2008
Combined	1240.5	Murie and Parkyn 2008
K		
Male	0.29	Murie and Parkyn 2008
Female	0.26	Murie and Parkyn 2008
Combined	0.28	Murie and Parkyn 2008
$t_0$		
Male	-0.92	Murie and Parkyn 2008
Female	-1.12	Murie and Parkyn 2008
Combined	-1.01	Murie and Parkyn 2008
Weight-length parameters		
a	$6.7 \times 10^{-8}$	Murie and Parkyn 2008
b	2.765	Murie and Parkyn 2008
Mortality		
M	0.25	NMFS 2006
F	0.2, 0.4, 0.6	NMFS 2006
D	0.2 (0.0, 0.4)	NMFS 2006
Proportion Legal		
MSL (commercial)	762	Hood 2006
MSL (recreational)	914.4	Hood 2006
$\sigma$	SL*0.1	Coggins et al. 2007; Pine et al. 2008; Tetzlaff et al. 2011
Fecundity		
$a_f$	655746	Harris et al. 2007
$b_f$	387.897	Harris et al. 2007
N	14	Harris et al. 2007
Fertility		
$f_{\max}$	0.8 (0.6, 1.0)	Jerez et al. 2006
$\Theta$	20 (80)	Heppell et al. 2006
Recruitment		
recK	10 (5, 15)	Meyers et al. 1999, Goodwin et al. 2006
$R_0$	$3.5 \times 10^5$ ( $3.0 \times 10^5$ , $4.0 \times 10^5$ )	Diaz et al. 2005

Table 3-2. Gear selectivities for Gulf of Mexico greater amberjack. CMHL = commercial handline, CMLL = commercial longline, HB = headboat, CB+PB = combined recreational charter and private fisheries. Values from Diaz et al. (2005).

Gear	Age									
	1	2	3	4	5	6	7	8	9	10+
CMHL	0.0	0.0	0.2	0.8	1.0	1.0	1.0	1.0	1.0	1.0
CMLL	0.0	0.0	0.0	0.5	0.9	1.0	1.0	1.0	1.0	1.0
HB	0.0	1.0	0.9	0.4	0.0	0.0	0.0	0.0	0.0	0.0
CB+PB	0.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 3-3. Proportion of mature male and female Gulf of Mexico greater amberjack by age. Female values from Murie and Parkyn (2008), and male values from D. Murie and D. Parkyn (University of Florida, unpublished data).

Sex	Age									
	1	2	3	4	5	6	7	8	9	10+
Male	0.103	0.103	0.597	0.804	0.806	1.000	1.000	1.000	1.000	1.000
Female	0.029	0.067	0.225	0.844	0.857	0.900	1.000	1.000	1.000	1.000

Table 3-4. Sensitivity analysis for Gulf of Mexico greater amberjack models with sex ratios applied at recruitment. Bold denotes a relevant percent change ( $\pm 10\%$  from the base case scenario at original value).

Input parameter	Original Value	Alternate Value 1	Alternate Value 2	Output parameter	% Change for alternate value 1	% Change for alternate value 2
D	0.2	0.0	0.4	MSSB	<b>34.89</b>	<b>-21.94</b>
				FSSB	<b>22.12</b>	<b>-31.75</b>
				EP	<b>31.04</b>	<b>-27.45</b>
				SP	<b>30.05</b>	<b>-27.58</b>
				FEP	<b>32.15</b>	<b>-26.69</b>
				wSPR	<b>24.36</b>	<b>-29.75</b>
				SPR	<b>13.60</b>	<b>-12.01</b>
				$f_{\max}$	0.8	0.6
FSSB	-1.18	0.14				
EP	-3.04	-3.81				
SP	-2.00	-4.97				
FEP	<b>-26.43</b>	<b>27.43</b>				
wSPR	-3.75	-4.04				
SPR	0.00	0.00				
$\Theta$	20	80	.			
				FSSB	0.76	.
				EP	-2.11	.
				SP	0.54	.
				FEP	1.22	.
				wSPR	-3.48	.
				SPR	0.00	.
				$R_0$	350000	300000
FSSB	<b>-13.93</b>	<b>13.25</b>				
EP	<b>-14.07</b>	<b>12.11</b>				
SP	<b>-16.74</b>	<b>12.90</b>				
FEP	<b>-13.14</b>	<b>12.63</b>				
wSPR	-5.52	-1.93				
SPR	0.00	0.00				
recK	10	5	15			
				FSSB	<b>-88.73</b>	<b>34.13</b>
				EP	<b>-88.52</b>	<b>38.06</b>
				SP	<b>-88.58</b>	<b>31.11</b>
				FEP	<b>-89.12</b>	<b>37.26</b>
				wSPR	<b>-88.98</b>	<b>32.88</b>
				SPR	0.00	0.00

Table 3-5. Sensitivity analysis for Gulf of Mexico greater amberjack models with sex ratios applied based on size. Bold denotes a relevant percent change ( $\pm 10\%$  from the base case scenario at original value).

Input parameter	Original value	Alternate value 1	Alternate value 2	Output parameter	% Change for alternative value 1	% Change for alternative value 2
D	0.2	0	0.4	MSSB	<b>23.88</b>	<b>-30.15</b>
				FSSB	<b>33.50</b>	<b>-32.75</b>
				EP	<b>24.56</b>	<b>-26.42</b>
				SP	<b>25.79</b>	<b>-24.37</b>
				FEP	<b>26.58</b>	<b>-30.61</b>
				wSPR	<b>27.92</b>	<b>-29.40</b>
				SPR	<b>14.08</b>	<b>-12.37</b>
				f <sub>max</sub>	0.8	0.6
FSSB	-4.11	-0.70				
EP	-1.13	-0.39				
SP	3.41	-1.74				
FEP	<b>-26.78</b>	<b>16.96</b>				
wSPR	4.59	1.24				
SPR	0.00	0.00				
Θ	-20	-80	.			
				FSSB	0.61	.
				EP	-5.40	.
				SP	-2.19	.
				FEP	-0.98	.
				wSPR	0.43	.
				SPR	0.00	.
				R <sub>0</sub>	350000	300000
FSSB	<b>-15.92</b>	<b>12.59</b>				
EP	<b>-15.90</b>	9.55				
SP	<b>-12.64</b>	<b>13.10</b>				
FEP	<b>-17.60</b>	<b>11.04</b>				
wSPR	0.40	-3.88				
SPR	0.00	0.00				
recK	10	5	15			
				FSSB	<b>-87.34</b>	<b>33.68</b>
				EP	<b>-87.17</b>	<b>33.94</b>
				SP	<b>-86.72</b>	<b>38.19</b>
				FEP	<b>-88.20</b>	<b>30.49</b>
				wSPR	<b>-88.00</b>	<b>40.75</b>
				SPR	0.00	0.00

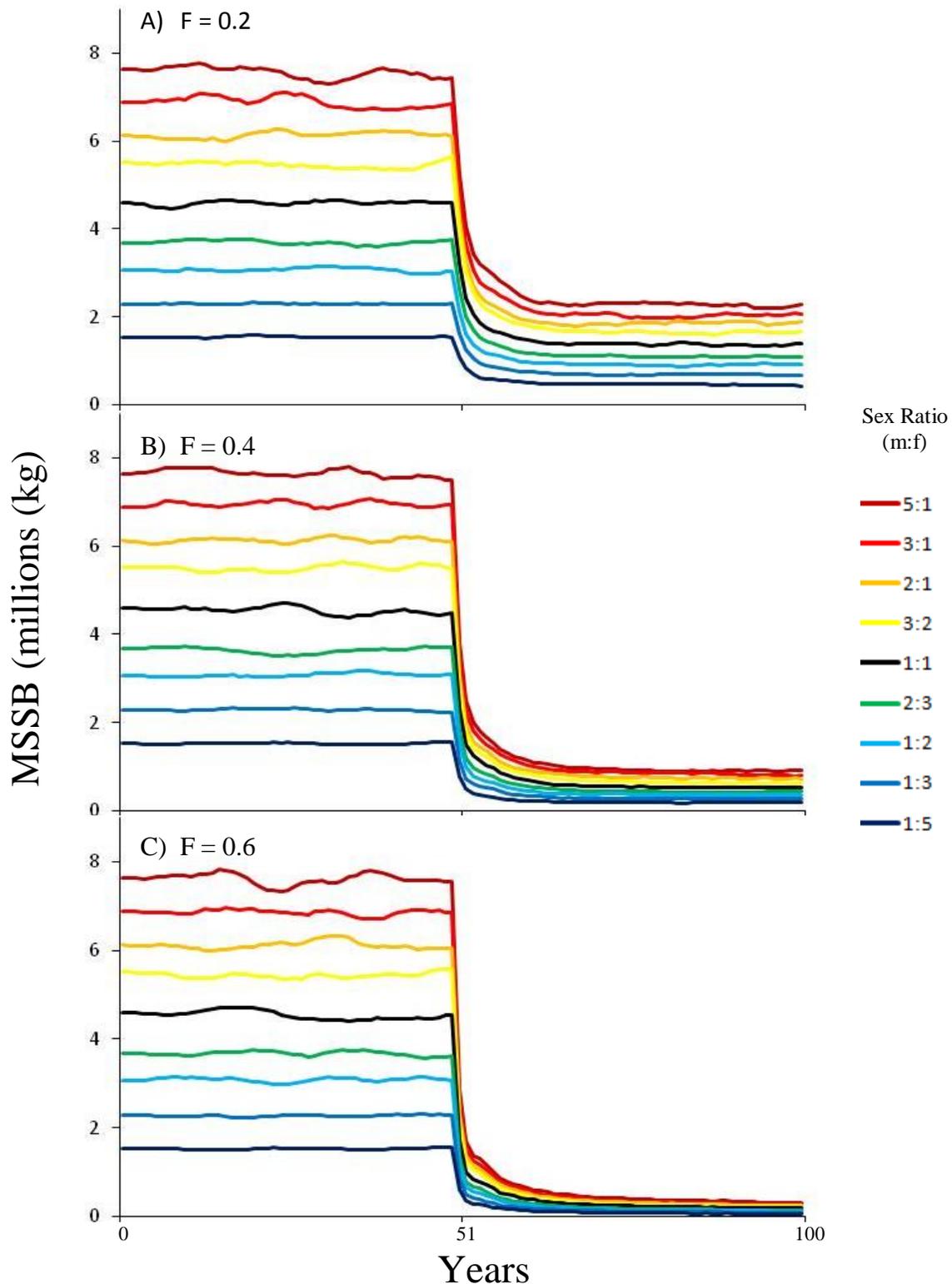


Figure 3-1. Male spawning stock biomass (MSSB) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at recruitment over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51.

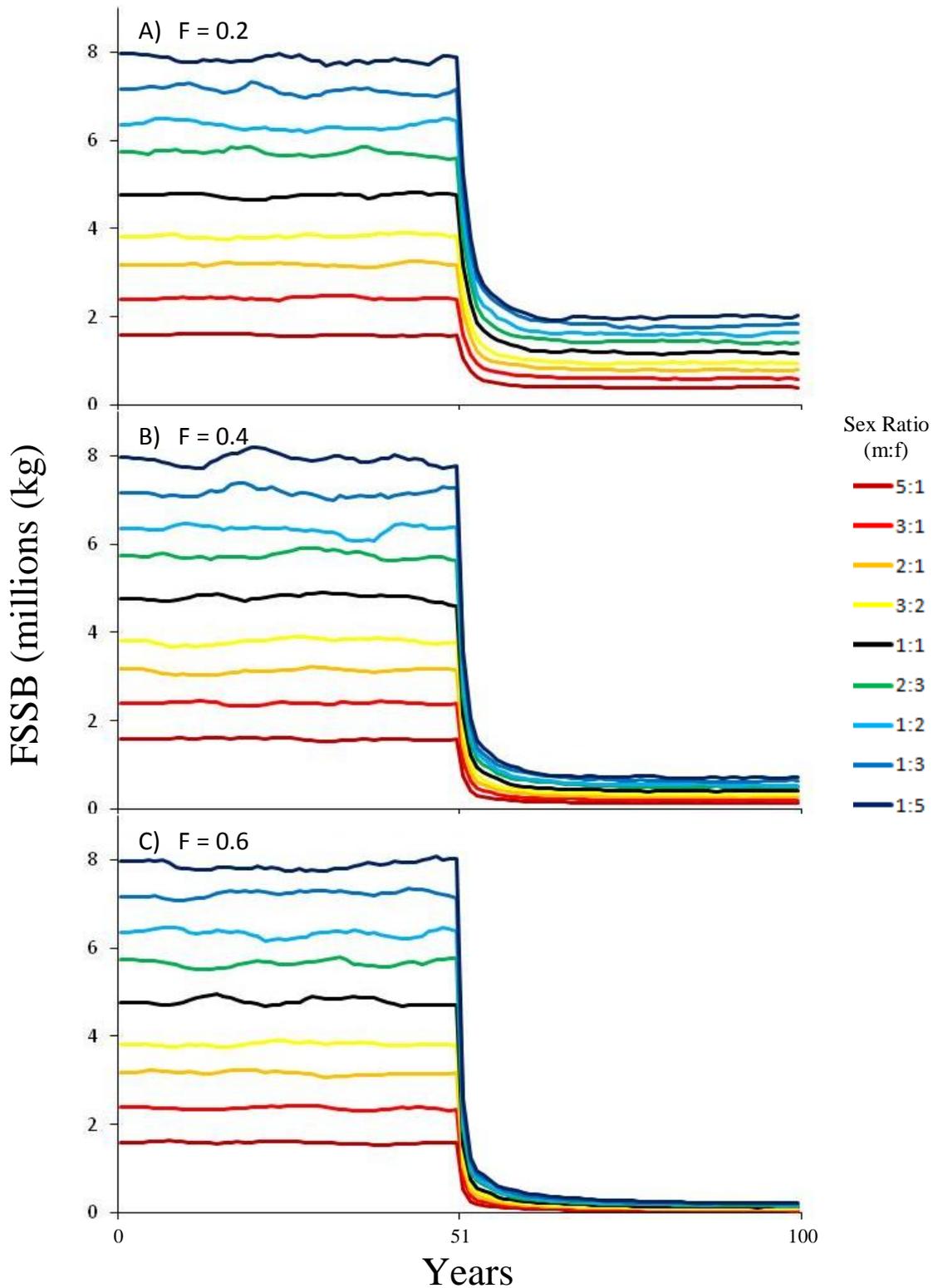


Figure 3-2. Female spawning stock biomass (FSSB) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at recruitment over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51.

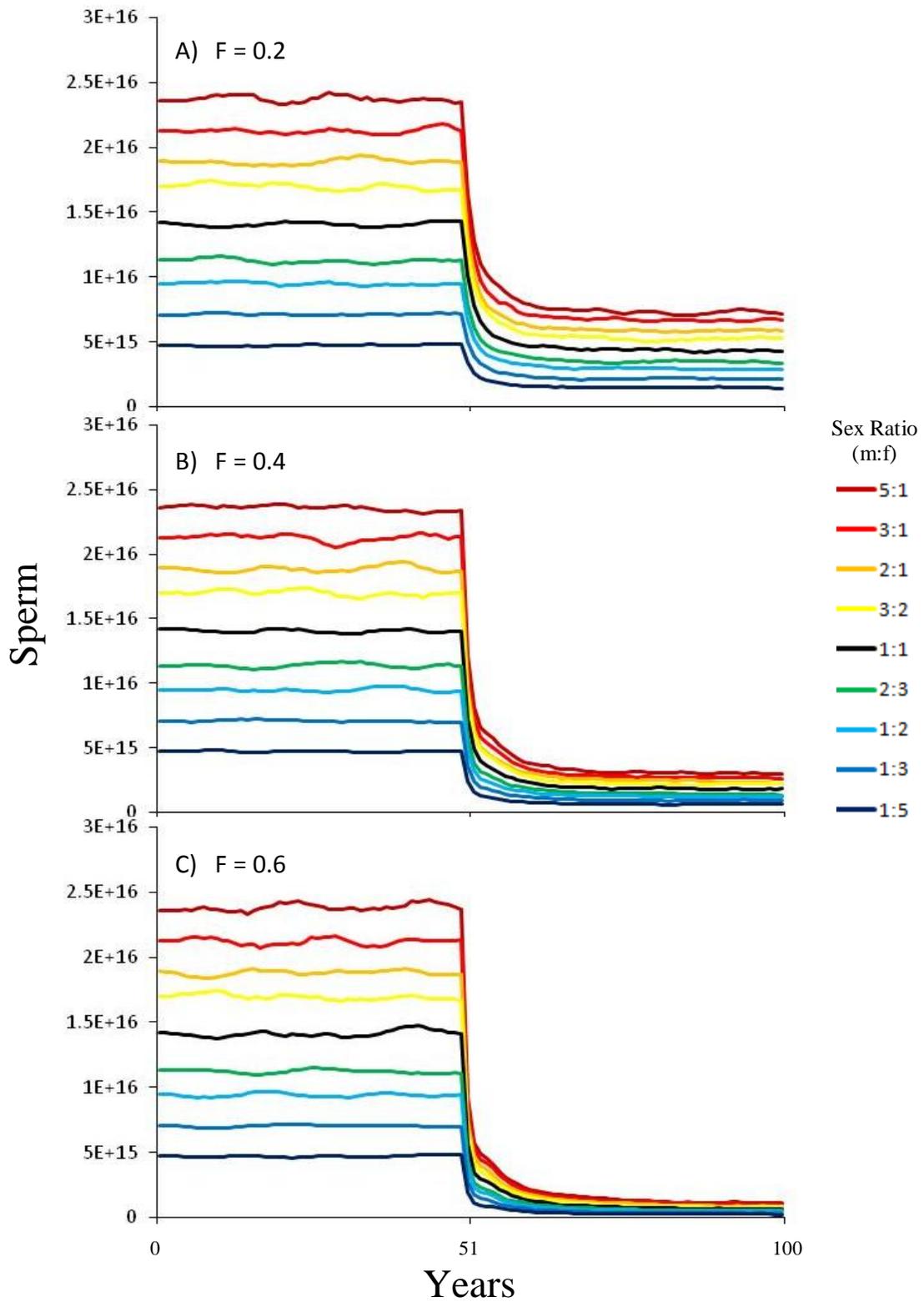


Figure 3-3. Total sperm production (SP) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at recruitment  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51.

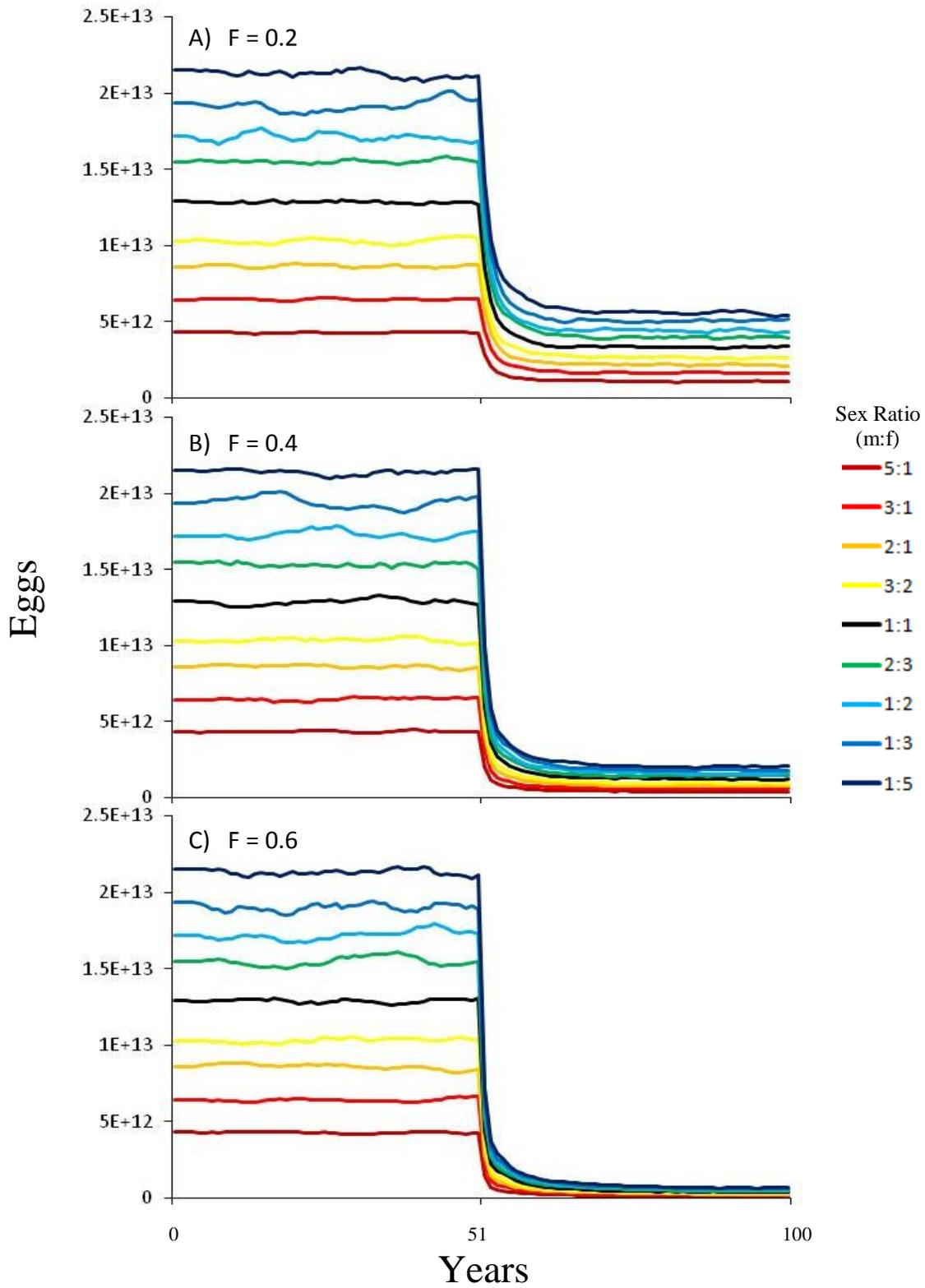


Figure 3-4. Total egg production (EP) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at recruitment over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51.

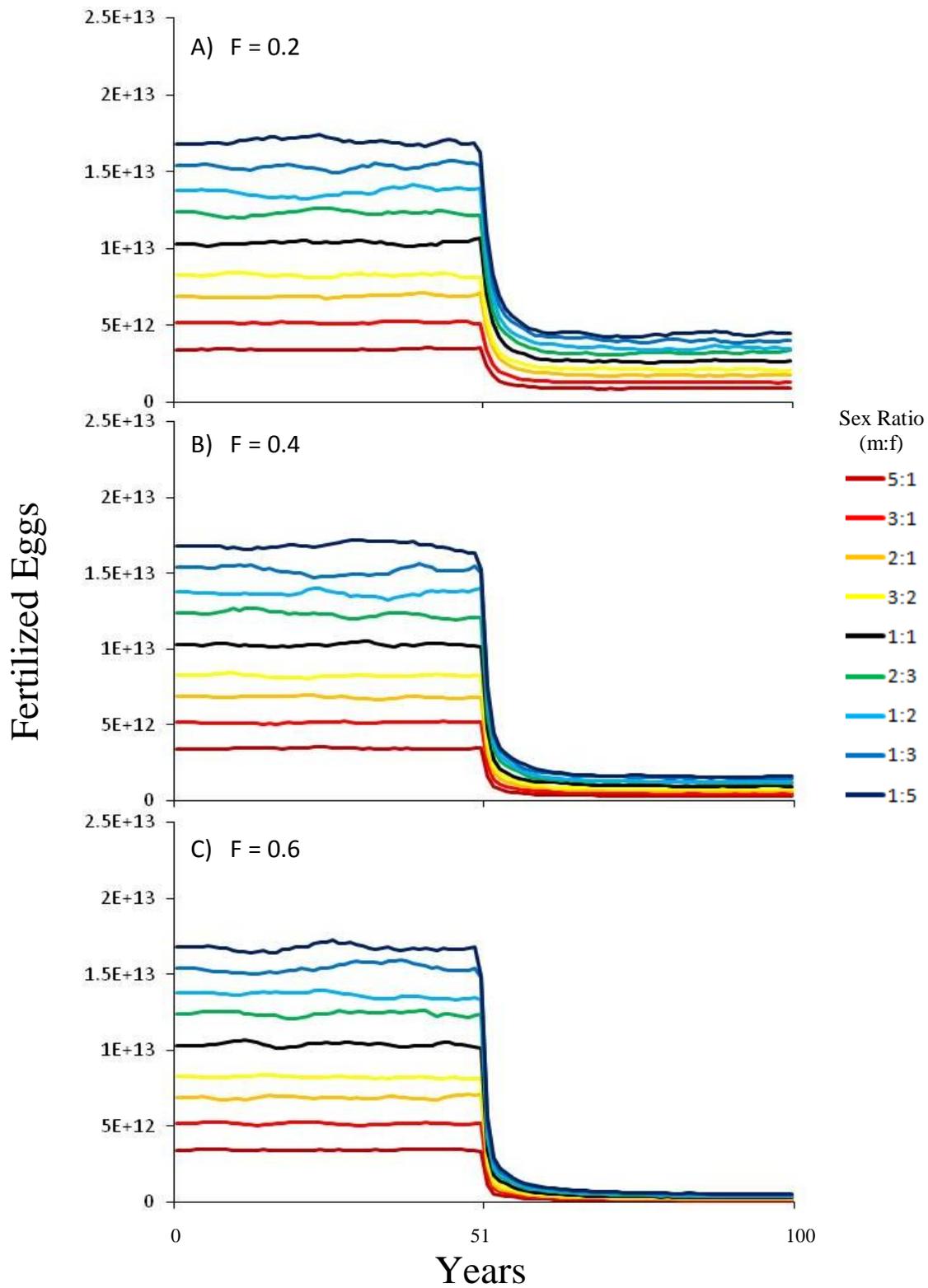


Figure 3-5. Total fertilized egg production (FEP) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at recruitment  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51.

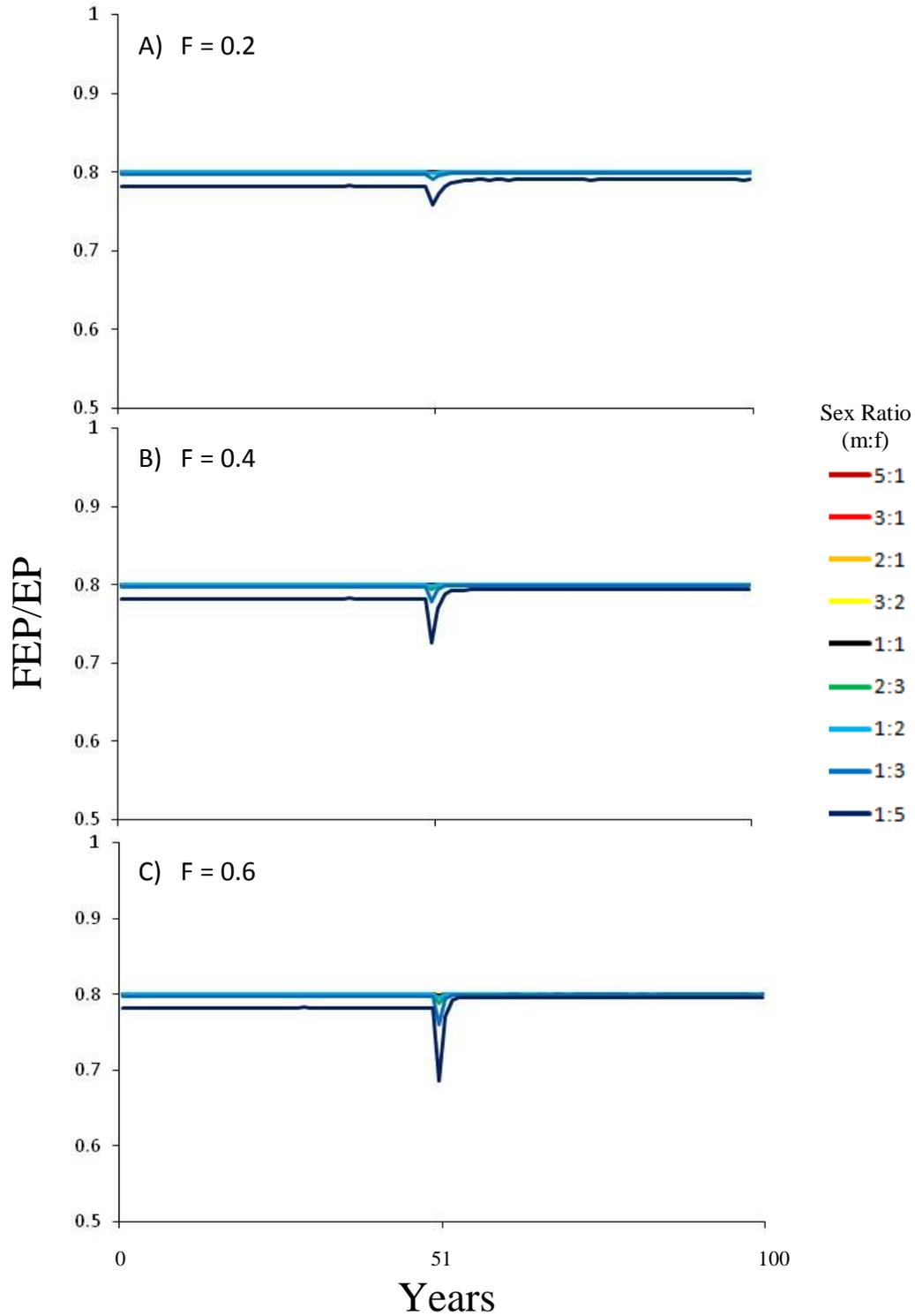


Figure 3-6. Ratio of fertilized egg production to egg production (FEP/EP) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at recruitment over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Values less than the maximum fertilization rate modeled (0.8) indicated potential sperm limitation. Fishing was initiated in year 51. All sex ratio are graphed, but most are stacked due to equivalent values of FEP/EP.

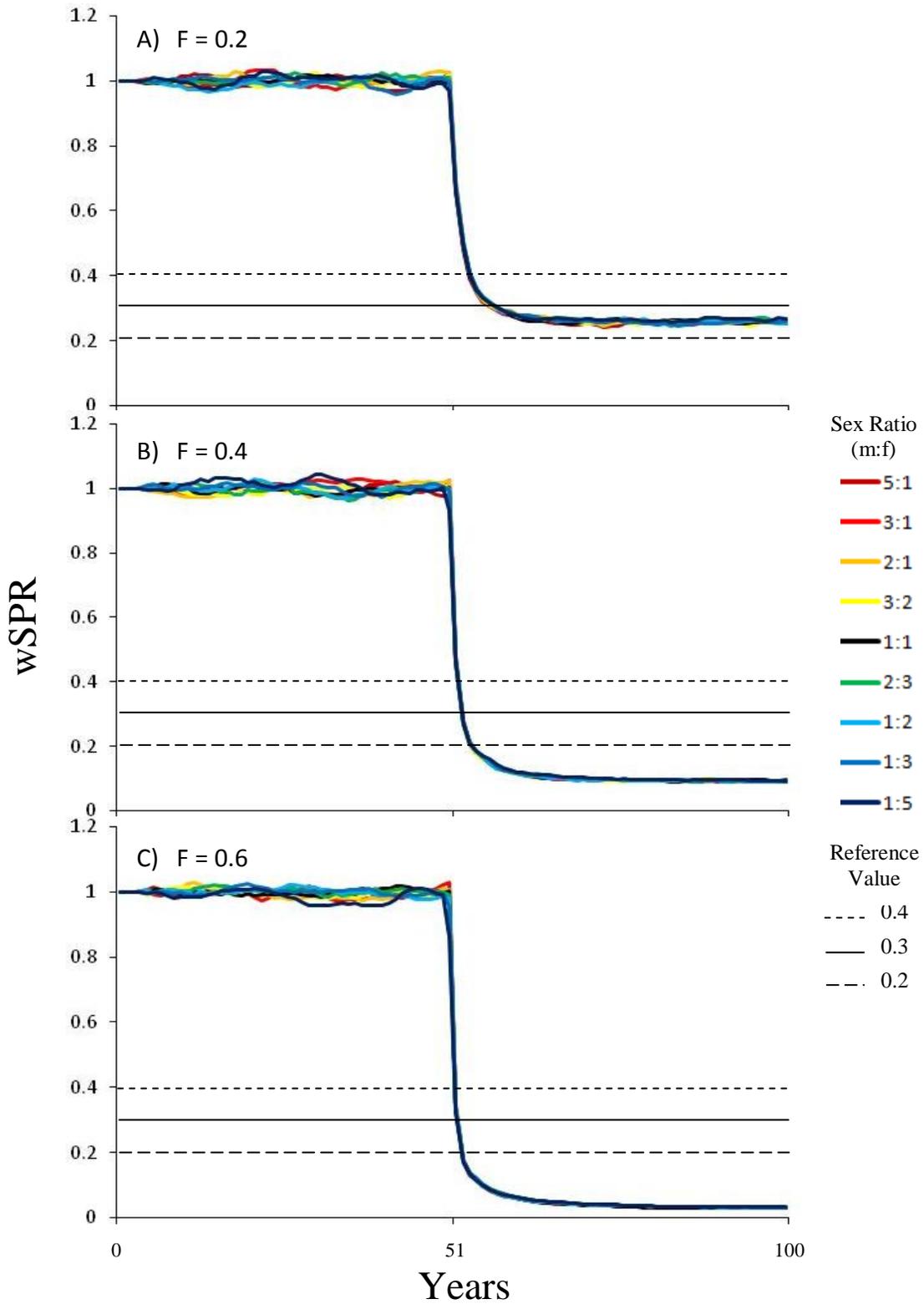


Figure 3-7. Weighted spawning potential ratio (wSPR) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at recruitment over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51. Horizontal lines represent wSPR reference values.

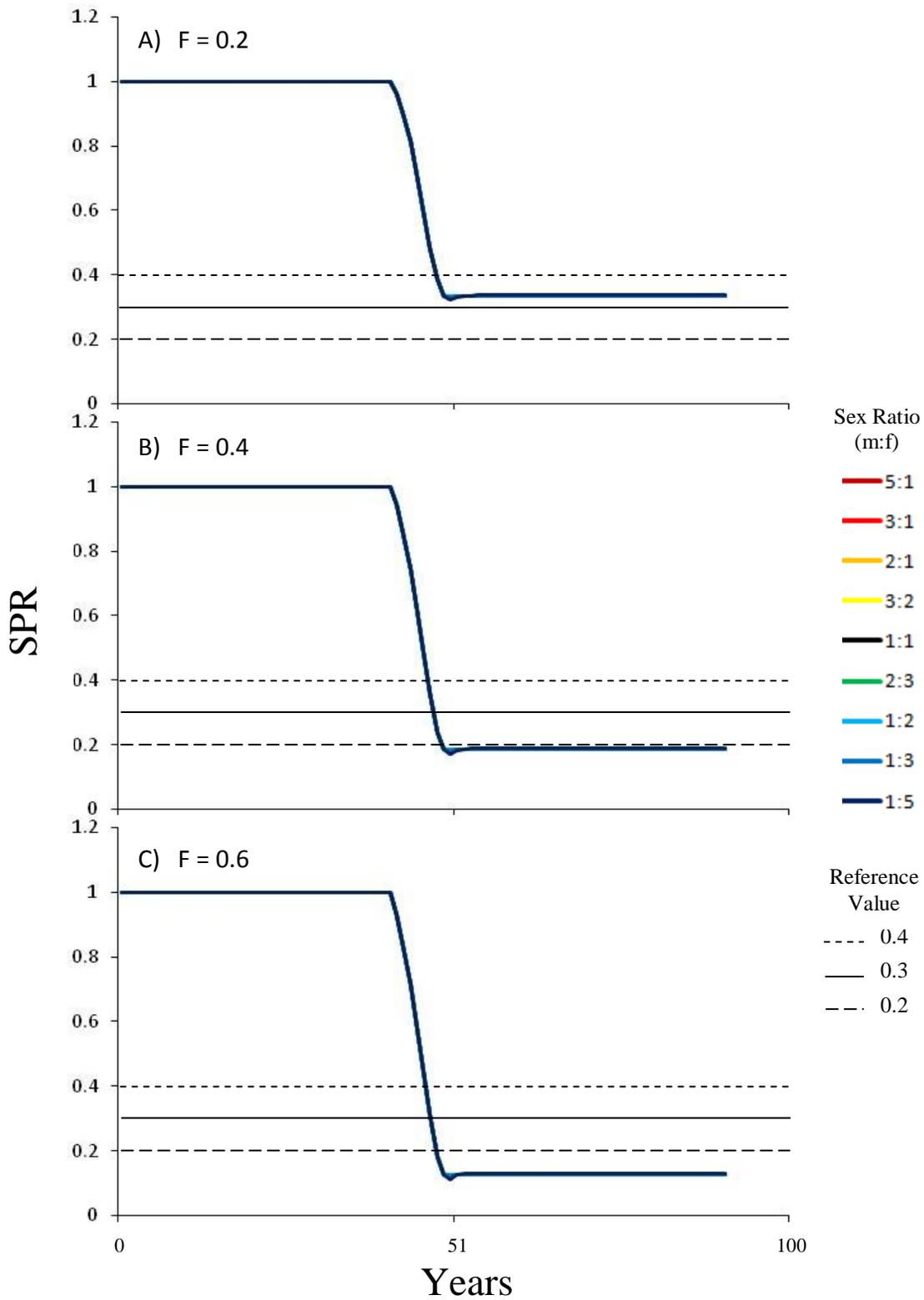


Figure 3-8. Spawning potential ratio (SPR) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at recruitment over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51. Horizontal lines represent SPR reference values.

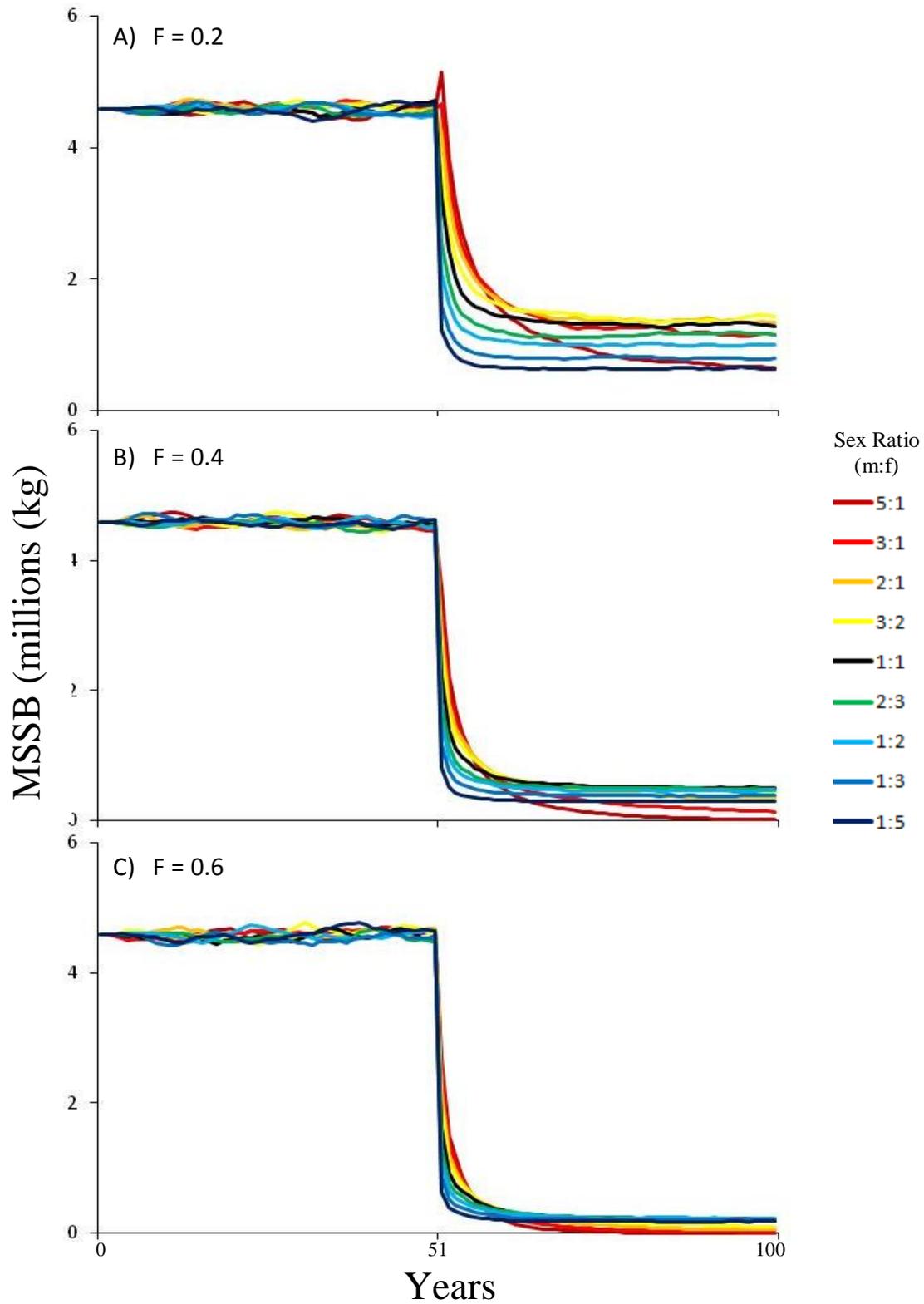


Figure 3-9. Male spawning stock biomass (MSSB) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at age 3 over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51.

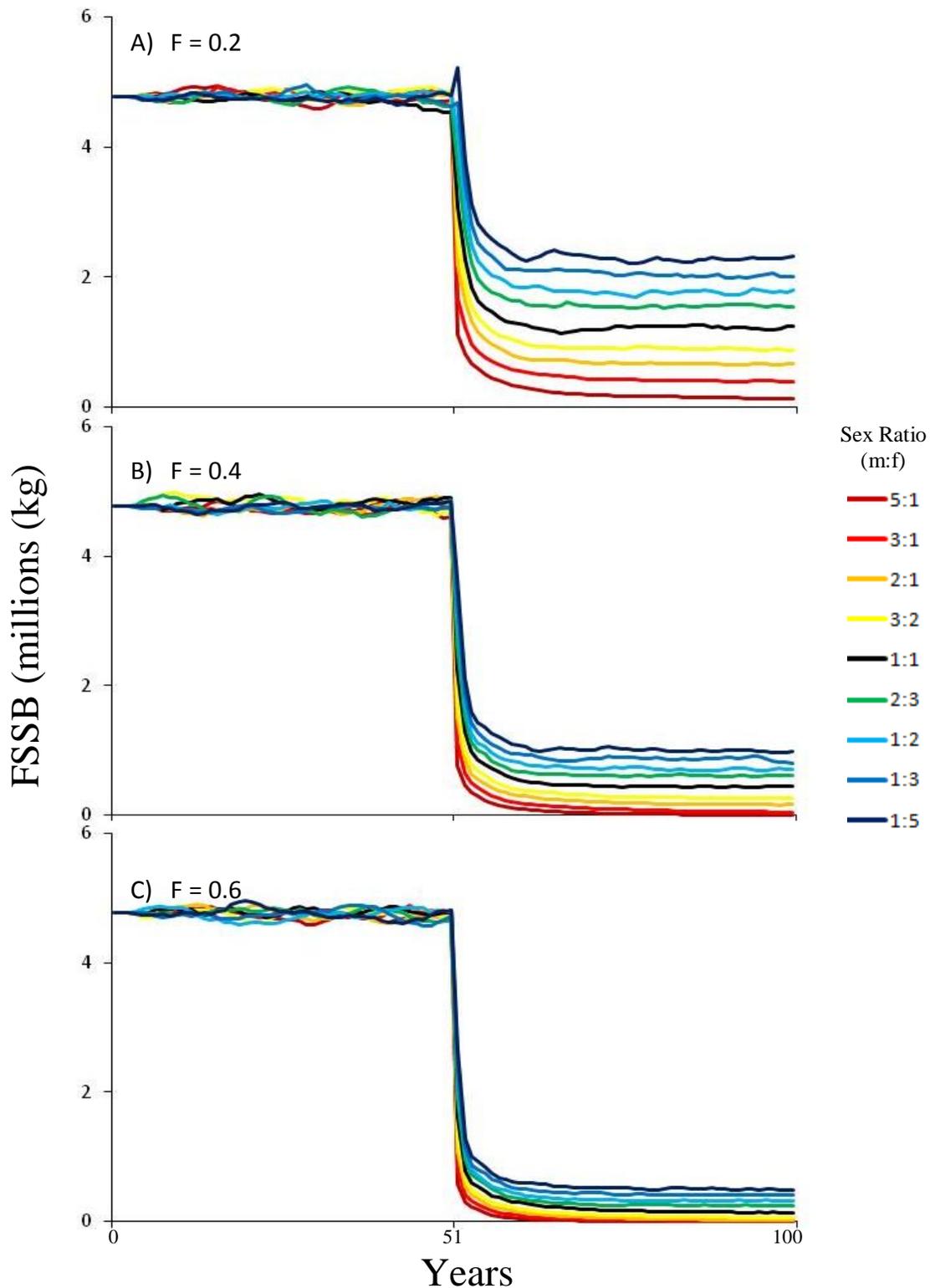


Figure 3-10. Female spawning stock biomass (FSSB) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at age 3 over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51.

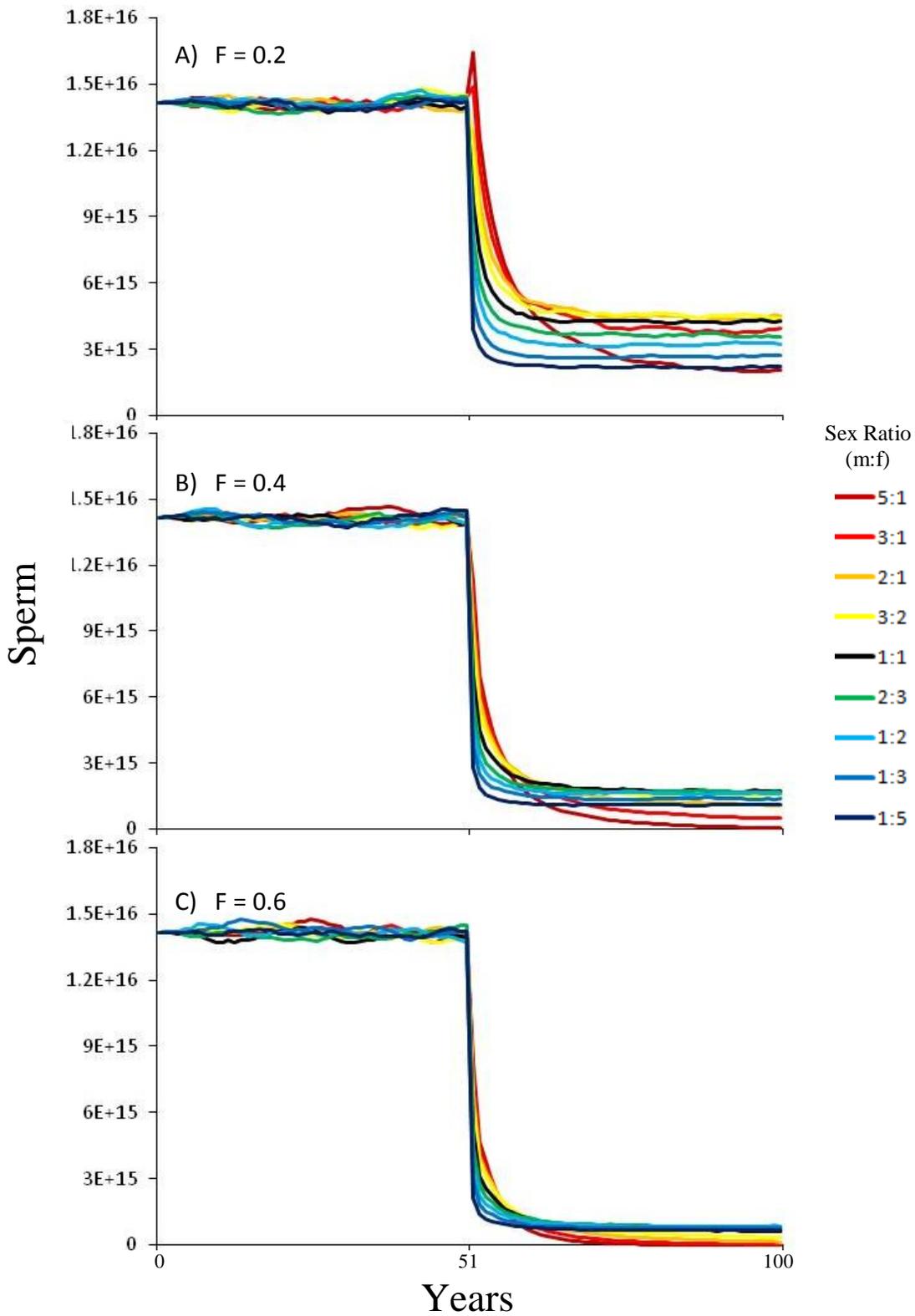


Figure 3-11. Total sperm production (SP) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at age 3 over  $F$ -values of 0.2 to 0.6. The base case sexratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51.

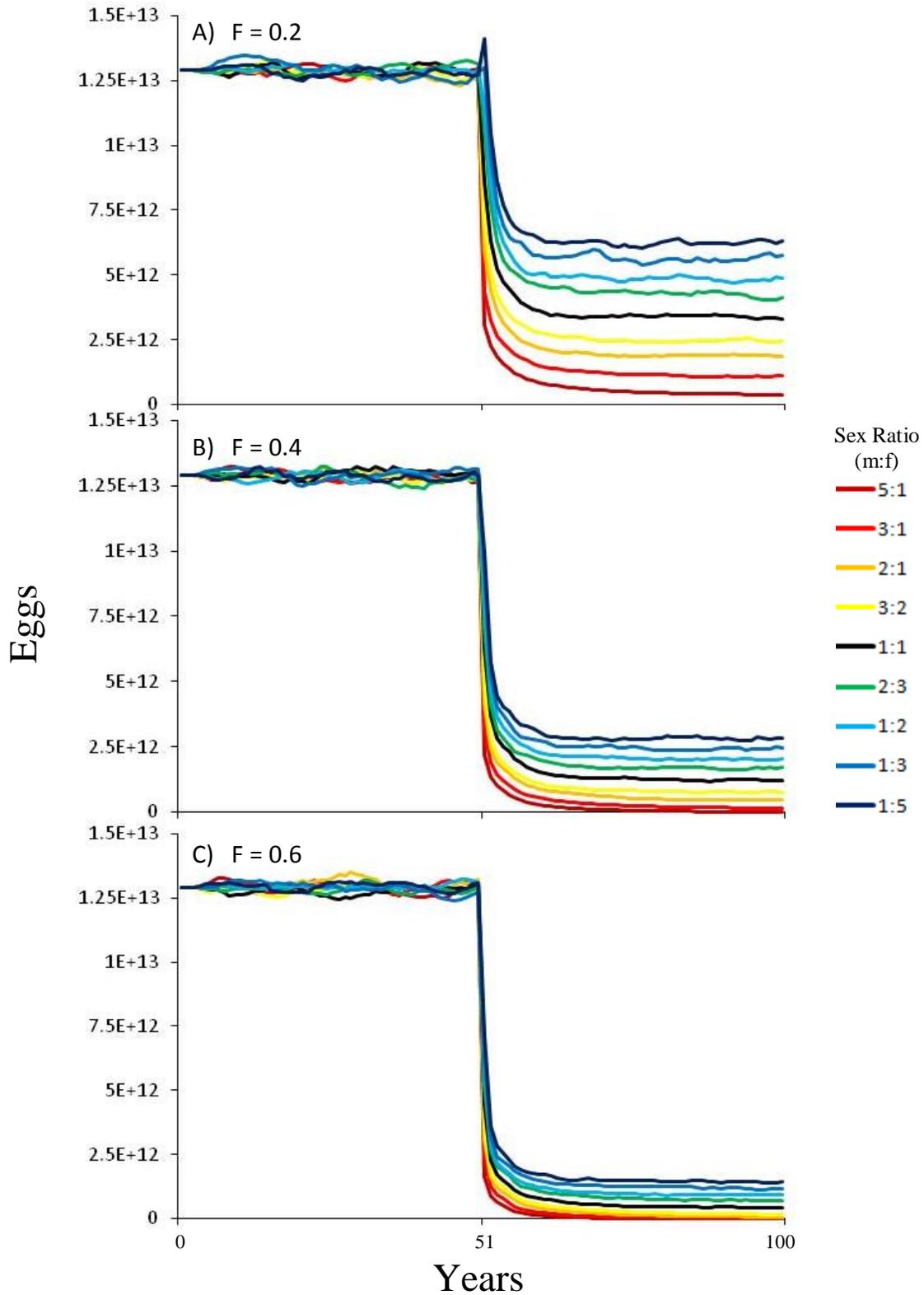


Figure 3-12. Total egg production (EP) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at age 3 over  $F$ -values of 0.2 to 0.6. The base case sexratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51.

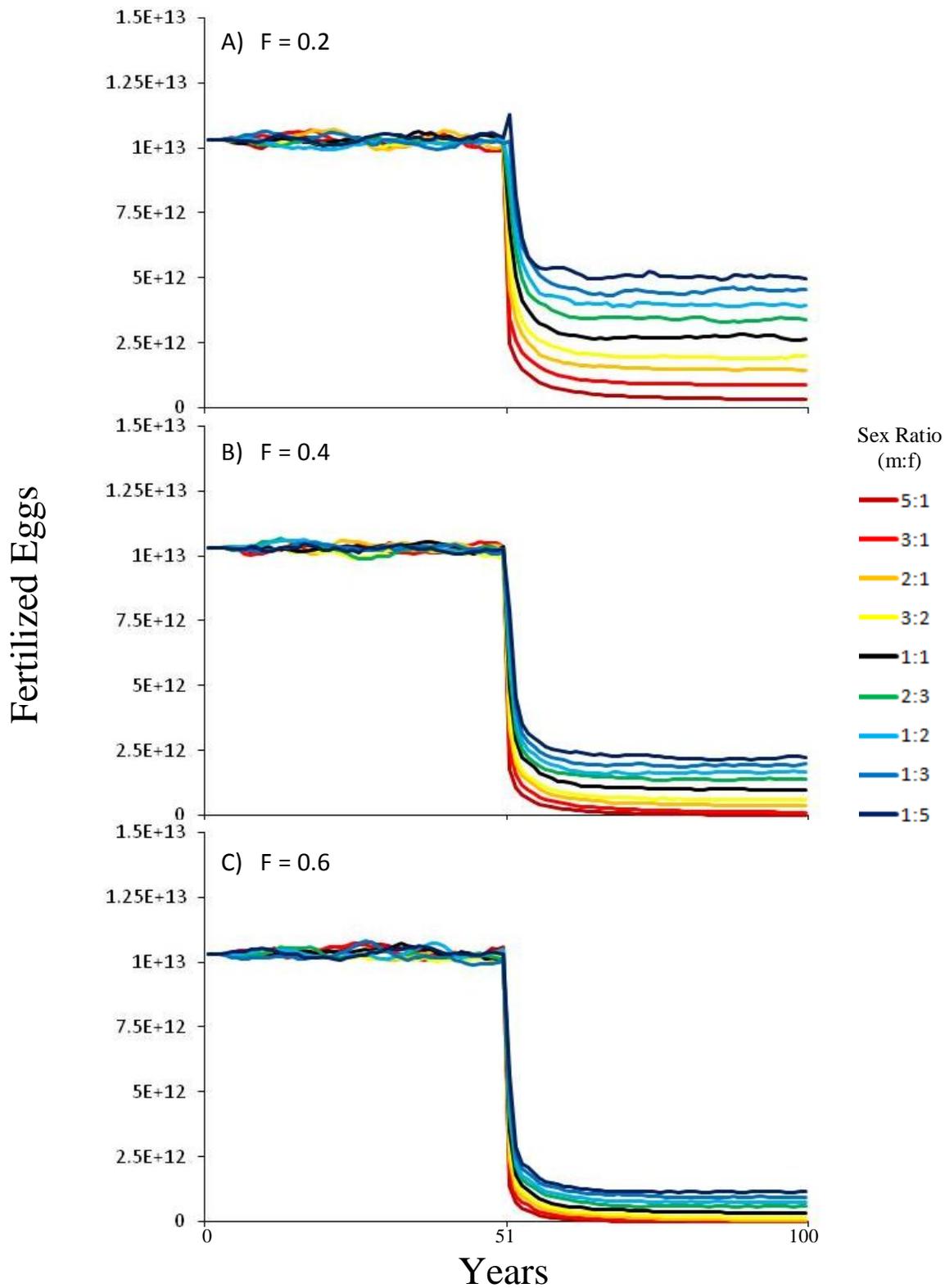


Figure 3-13. Total fertilized egg production (FEP) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at age 3 over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51.

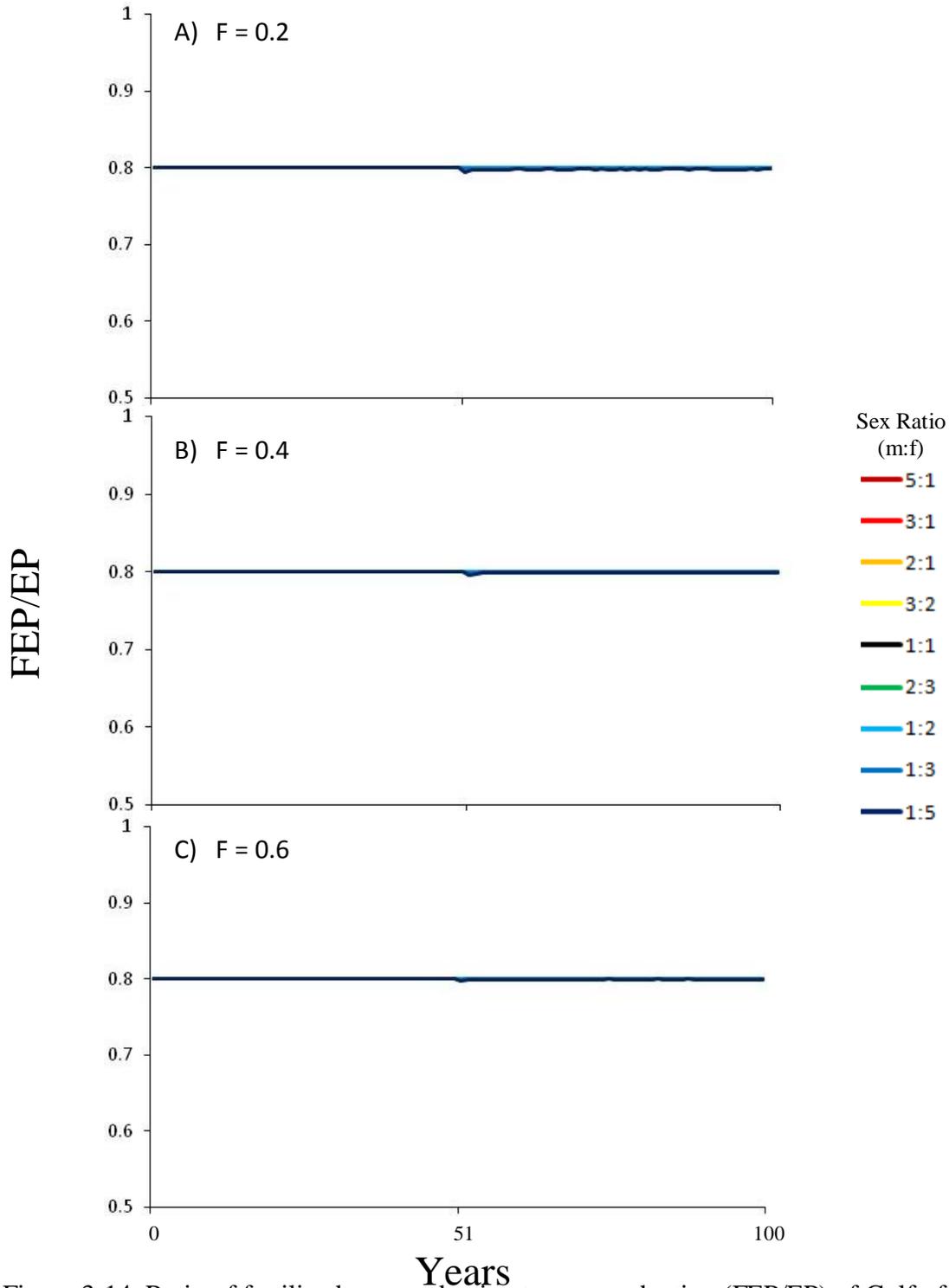


Figure 3-14. Ratio of fertilized egg production to egg production (FEP/EP) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at age 3 over a  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Values less than the maximum fertilization rate modeled (0.8) indicated the potential for sperm limitation. Fishing was initiated in year 51. All sex ratios are graphed, but are stacked due to equivalent values of FEP/EP.

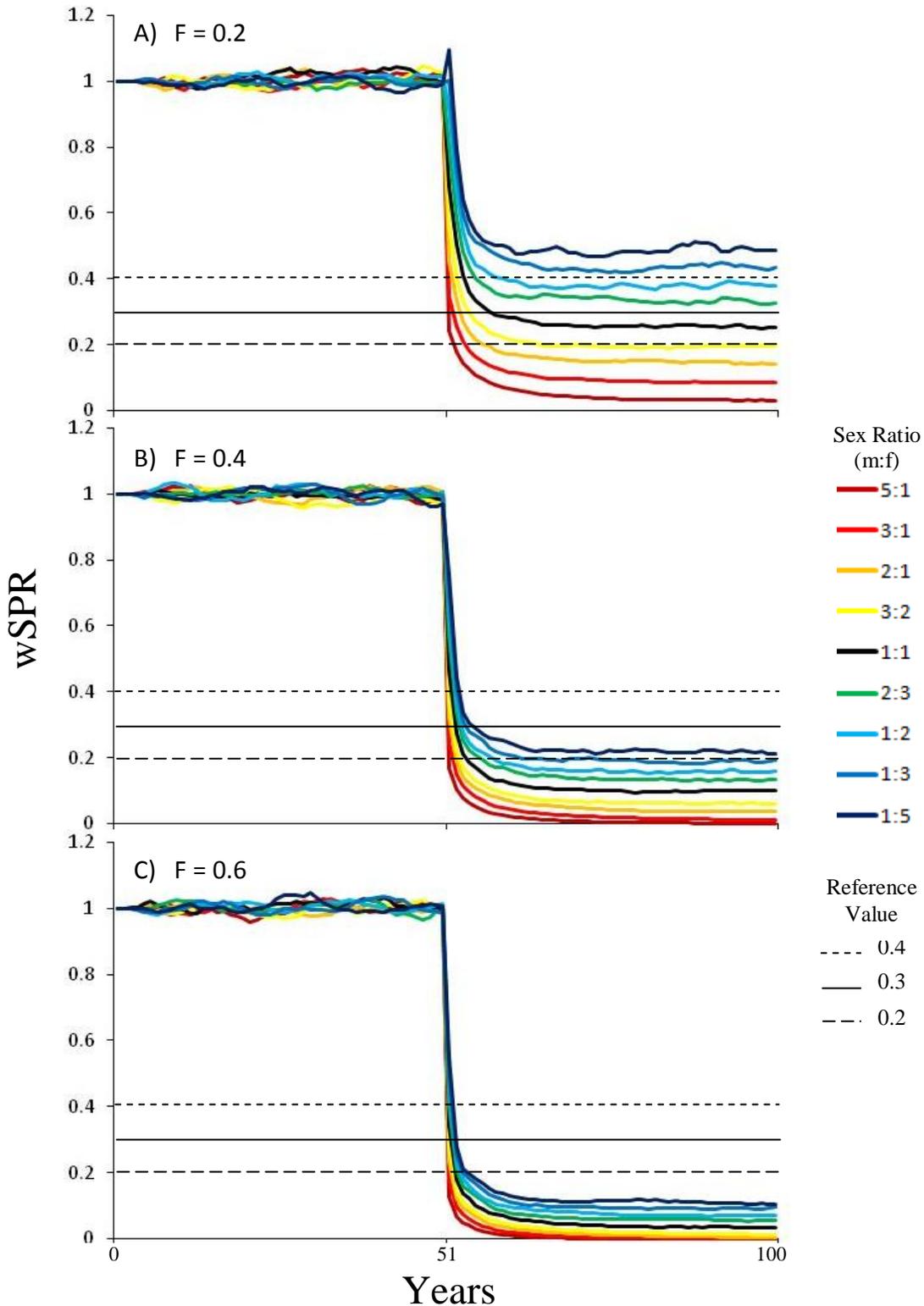


Figure 3-15. Weighted spawning potential ratio (wSPR) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at age 3 over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51. Horizontal lines represent wSPR reference values.

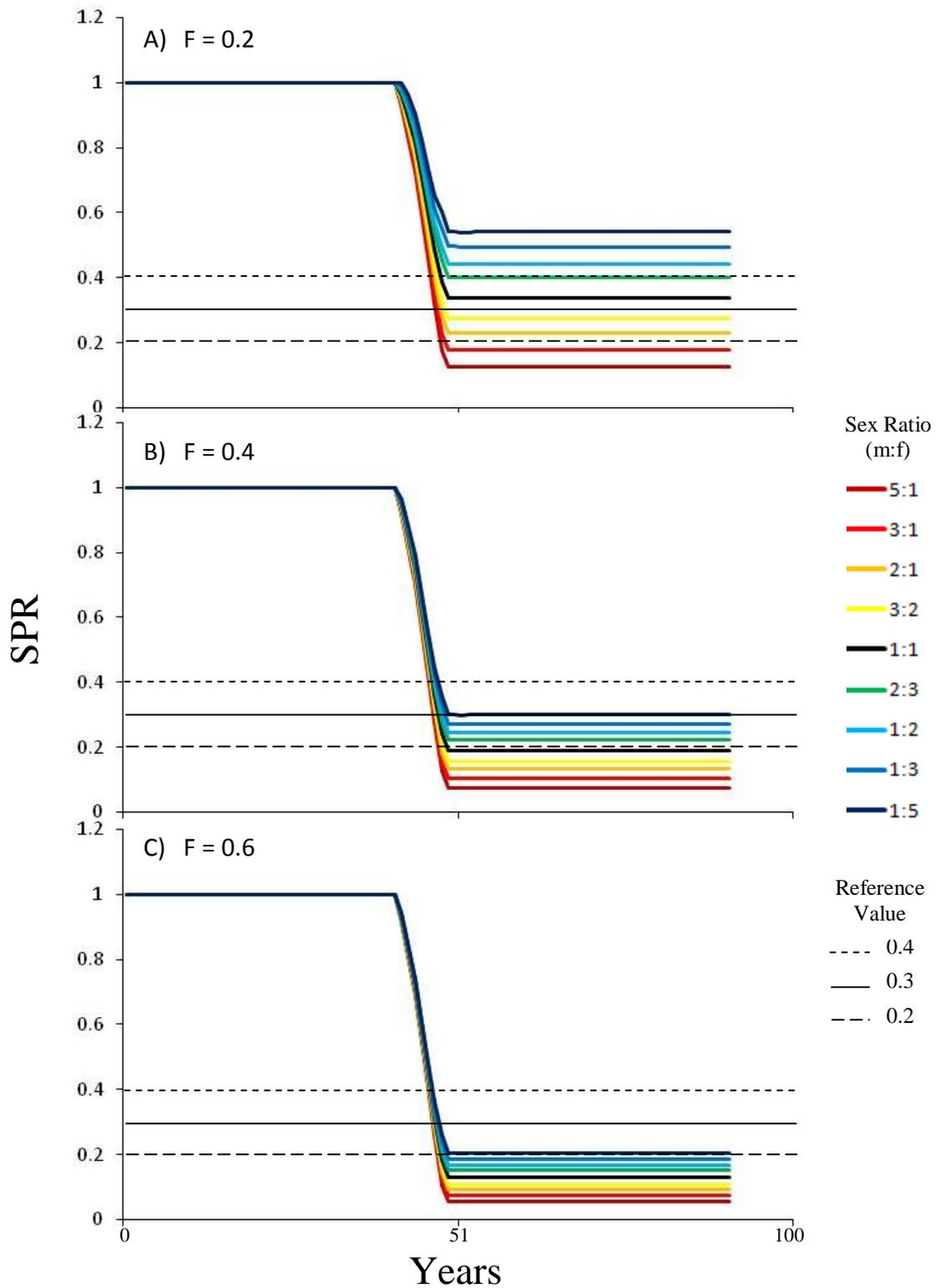


Figure 3-16. Spawning potential ratio (SPR) of Gulf of Mexico greater amberjack for models with a range of sex ratios applied at age 3 over  $F$ -values of 0.2 to 0.6. The base case sex ratio was 1:1 and the base case  $F$  was 0.4. Fishing was initiated in year 51. Horizontal lines represent SPR reference values.

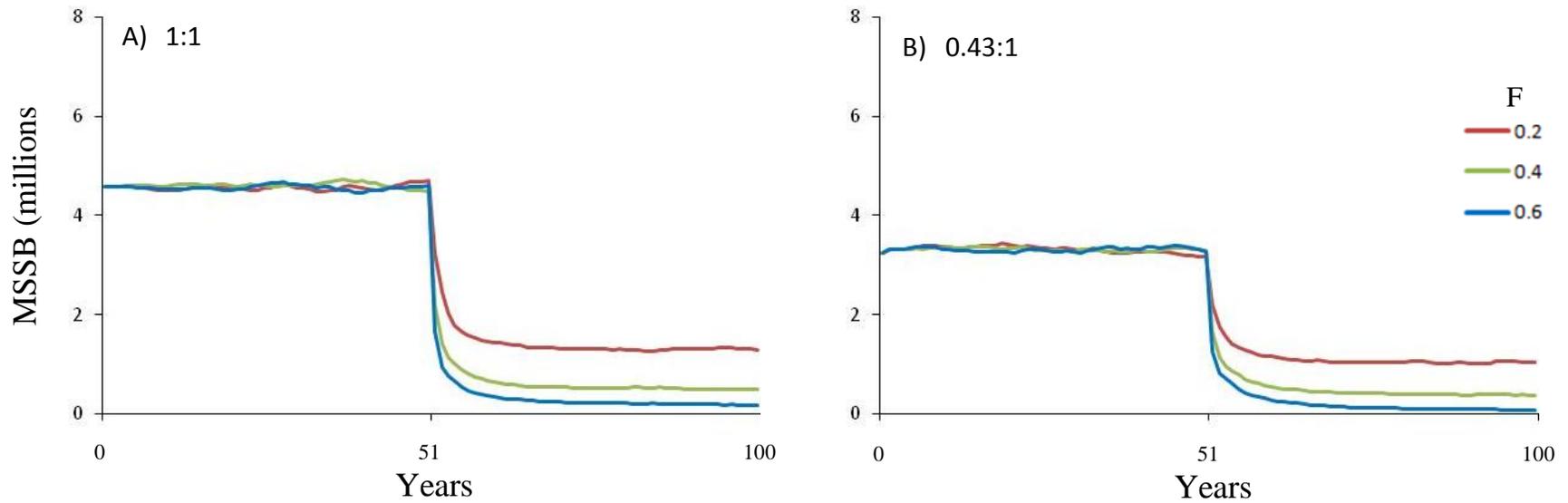


Figure 3-17. Male spawning stock biomass (MSSB) for Gulf of Mexico greater amberjack across a range of  $F$ -values (0.4 = base case) for A) the base case 1:1 sex ratio; B) and a 0.43:1 female-skewed sex ratio for fish > 1 m fork length. Fishing was initiated in year 51.

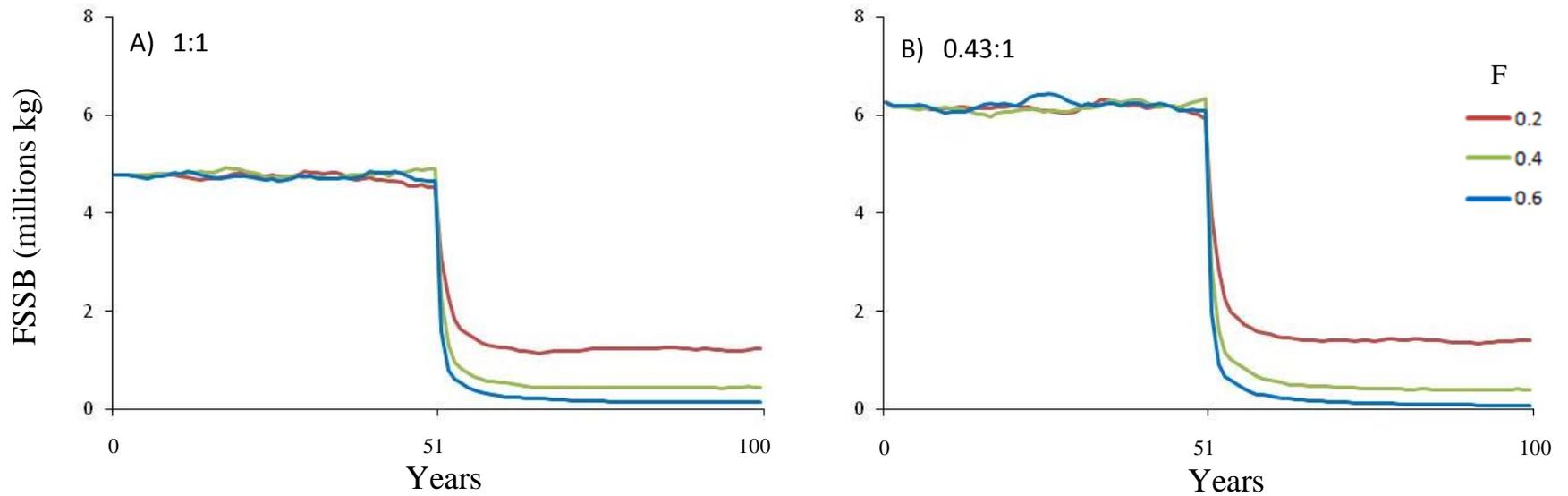


Figure 3-18. Female spawning stock biomass (FSSB) for Gulf of Mexico greater amberjack across a range of  $F$ -values (0.4 = base case) for A) the base case 1:1 sex ratio; and B) a 3:7 female-skewed sex ratio for fish > 1 m fork length. Fishing was initiated in year 51.

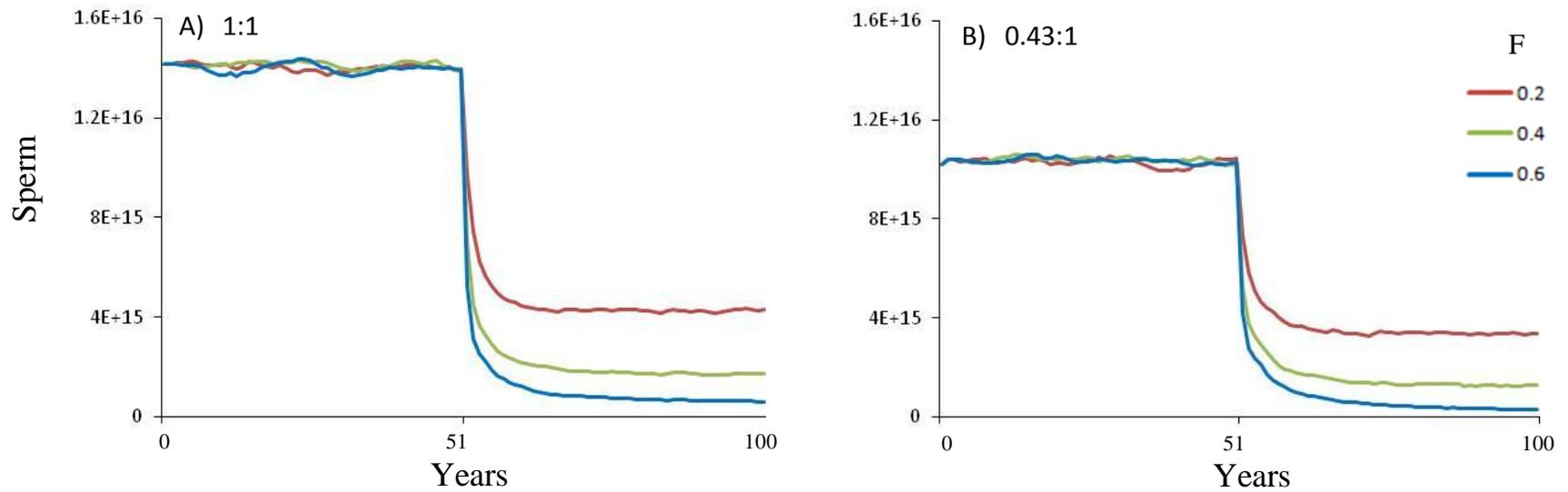


Figure 3-19. Total sperm production (SP) for Gulf of Mexico greater amberjack across a range of  $F$ -values (0.4 = base case) for A) the base case 1:1 sex ratio; and B) a 0.43:1 female-skewed sex ratio for fish > 1 m fork length. Fishing was initiated in year 51.

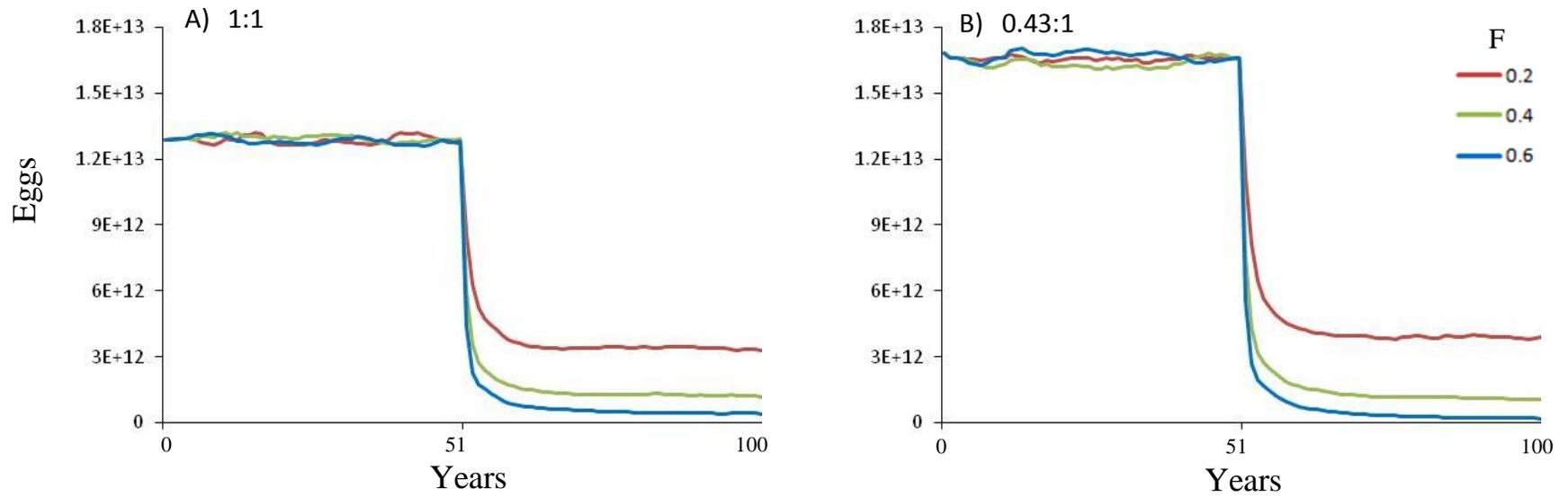


Figure 3-20. Total egg production (EP) for Gulf of Mexico greater amberjack across a range of  $F$ -values (0.4 = base case) for A) the base case 1:1 sex ratio; and B) a 0.43:1 female-skewed sex ratio for fish > 1 m fork length. Fishing was initiated in year 51.

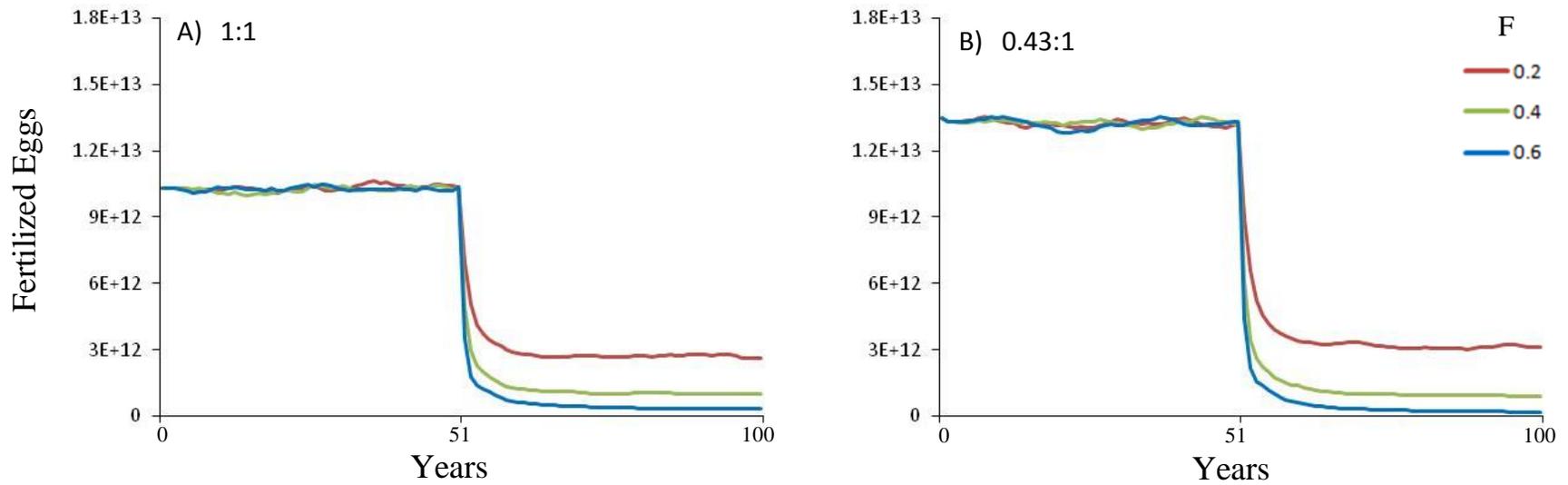


Figure 3-21. Total fertilized egg production (FEP) for Gulf of Mexico greater amberjack across a range of  $F$ -values ( $0.4 = \text{base case}$ ) for A) the base case 1:1 sex ratio; and B) a 0.43:1 female-skewed sex ratio for fish  $> 1$  m fork length. Fishing was initiated in year 51.

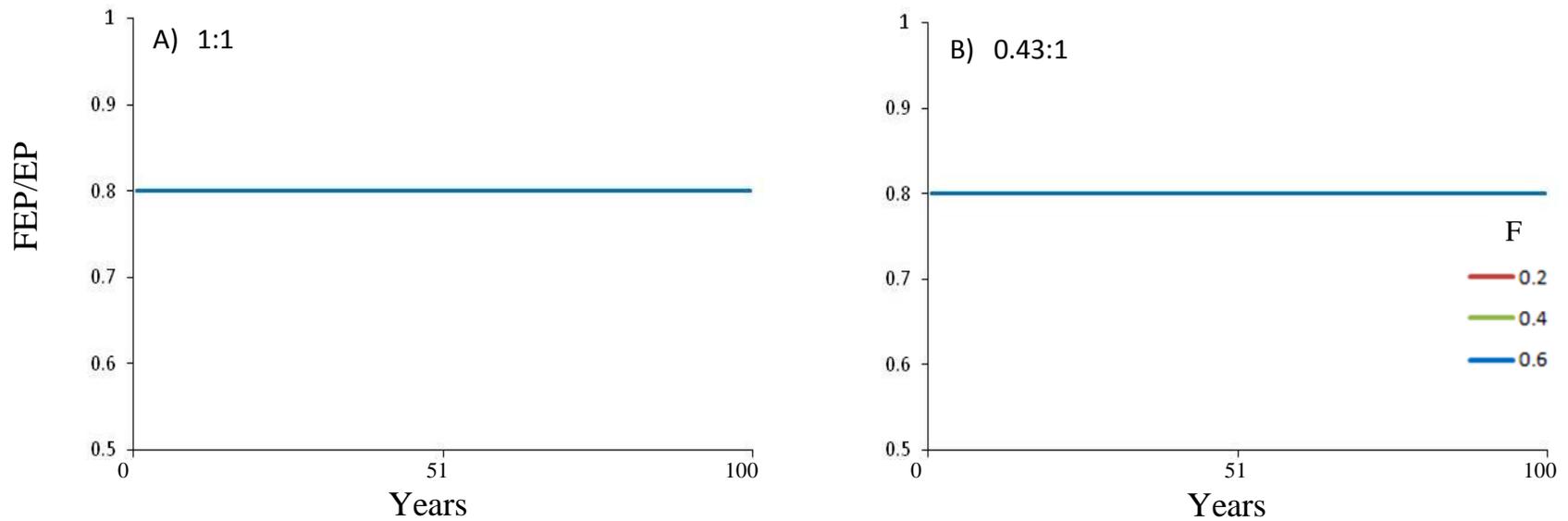


Figure 3-22. Ratio of fertilized egg production to egg production for Gulf of Mexico greater amberjack across a range of  $F$ -values (0.4 = base case) for A) the base case 1:1 sex ratio; and B) a 0.43:1 female-skewed sex ratio for fish > 1 m fork length. Values less than the maximum fertilization rate modeled (0.8) indicated the potential for sperm limitation. Fishing was initiated in year 51. All  $F$ -values are graphed but are stacked due to equivalent values of FEP/EP.

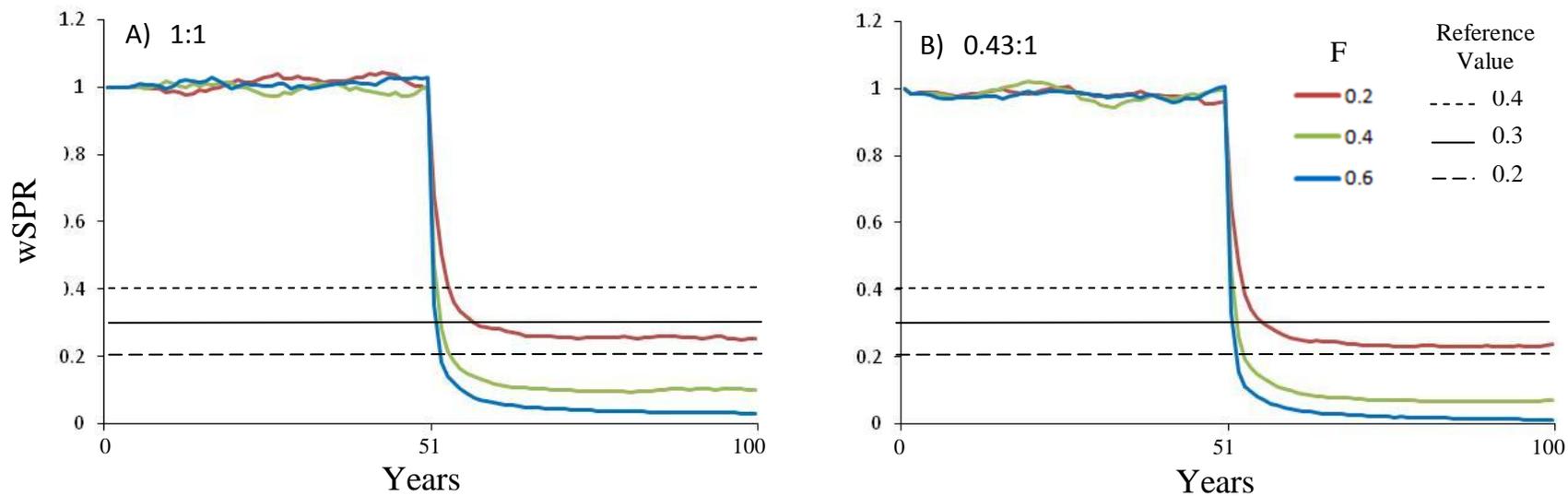


Figure 3-23. Weighted spawning potential ratio (wSPR) for Gulf of Mexico greater amberjack across a range of  $F$ -values (0.4 = base case) for A) the base case 1:1 sex ratio; and B) a 0.43:1 female-skewed sex ratio for fish > 1 m fork length. Fishing was initiated in year 51. Horizontal lines represent wSPR reference values.

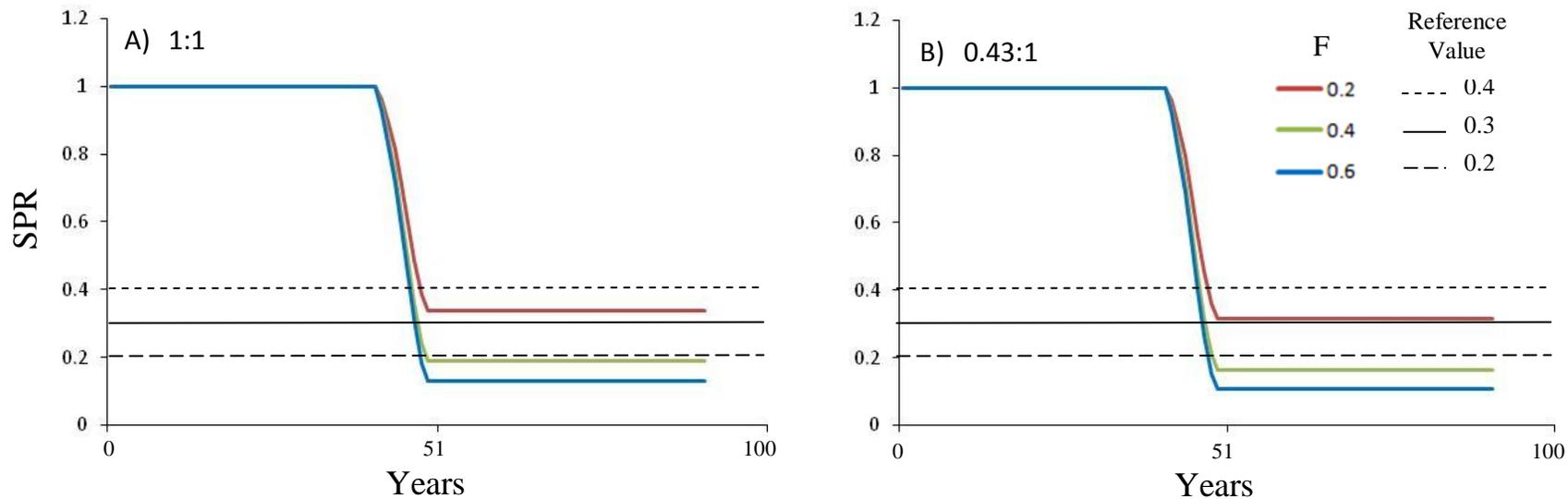


Figure 3-24. Spawning potential ratio (SPR) for Gulf of Mexico greater amberjack across a range of  $F$ -values (0.4 = base case) for A) the base case 1:1 sex ratio; and B) a 0.43:1 female-skewed sex ratio for fish > 1 m fork length. Fishing was initiated in year 51. Horizontal lines represent SPR reference values.

## CHAPTER 4 CONCLUSION

Information related to reproduction and sex-specific parameters in the Gulf of Mexico greater amberjack stock, which is considered to be overfished and undergoing overfishing, is still in need of further study. The development of a non-lethal means of sex determination for this species will allow for a considerable increase in our knowledge of sex-specific mortality and migration patterns, as well as determining more accurate sex ratios. Such information will allow for more well informed stock assessments and better management of this stock.

The use of external urogenital features has been used to non-lethally sex fish for a number of years, and in general the results of studies applying this method have yielded high accuracies. However, advances in technology and the relatively simple nature of this method have likely resulted in it falling to the wayside in many cases, with a more technologically advanced method being used instead. In some instances, a simple yet accurate method may actually be desirable, as is the case with non-lethally sexing a relatively large species that is to be immediately released while at sea. The use of external urogenital features requires no anesthesia, minimal costs and training, and is relatively rapid to perform, thus making it a good choice for application in attempting to sex such a species (greater amberjack in this case) at sea. The results of this study have shown that the use of external urogenital features is indeed an accurate means by which the sex of greater amberjack can be determined non-lethally as accuracies remained high regardless of the sex or size of an individual. Although reproductively active fish are by far the easiest to sex using this method, non-reproductively active individuals can also be sexed with relative ease. However, the utility of this method is likely diminished at a lower size threshold based on the small size of the urogenital pores (i.e., no amberjack <500 mm FL were sexed in this study).

Urogenital catheterization is another commonly used method for non-lethally sexing fish. Again, this is a relatively simple method requiring no anesthesia, minimal costs and training, and it is relatively rapid. This method has previously been used to determine the maturation state of greater amberjack in an aquaculture setting, and in this study it proved to be effective in verifying sexes determined by the use of external urogenital features and assessing the relative maturity of female fish.

The ability to non-lethally sex greater amberjack would add a great deal to our knowledge of the species if applied in tag and release studies, on-board observer programs, and scientific surveys. The combination of the two previously mentioned methods could allow for determination of sex-specific mortality rates, growth rates, and migration patterns, as well as better estimates of sex ratios, both overall and regionally. In addition, these methods could be used in an attempt to locate areas of spawning aggregations.

The information relating to reproduction and sex-specific parameters can be used to better understand the population dynamics of the Gulf of Mexico greater amberjack stock and lead to better management. This study modeled the potential impacts of sex ratios on the population dynamics of this stock. The sex ratios modeled were based on data from both lethal (port sampling, age and growth studies, etc.) and non-lethal (tag and release study) studies. The results of this study indicated that sex ratios can play a significant role in the estimation of productivity of greater amberjack. Even moderate male-skewing of the sex ratio of the individuals remaining in the Gulf stock can lead to a large decrease in productivity at current fishing mortality rates, while female-skewing could impart some resilience to fishing. The differences in productivity between male- and female-skewed and un-skewed populations were decreased with increasing fishing pressure. The female-skewing that was observed in the largest size classes of this species

also imparted some resilience to fishing pressure, but this resilience was quickly diminished upon the onset of fishing as the largest individuals were fished out. Sexually dimorphic growth could lead to sex-specific exploitation, which may lead to an alteration of the overall sex ratio. The current stock assessment for Gulf of Mexico greater amberjack assumes a 1:1 sex ratio. However, there was some evidence that the overall sex ratio of landings was moderately female-skewed, which could either indicate that the overall sex ratio for the Gulf of Mexico greater amberjack stock was female-skewed or that the female-skewing in the landings could be creating an overall sex ratio that was male-skewed. Site-specific sex ratios can also be highly skewed, but no clear patterns have emerged. Further research may reveal sex-specific migration patterns and regional skewing in sex ratios that could lead to further sex-specific exploitation. Knowledge that skewing of sex ratios can greatly affect the productivity of this species should be taken into account in its stock assessment. An erroneous sex ratio assumption (i.e., 1:1) could result in incorrect conclusions being made about its current stock status. A stock whose remaining population is more female-skewed than is assumed may actually not be as exploited as would be concluded, while a stock with a remaining population that is more male-skewed than is assumed is likely to be more exploited than would be concluded. With this in mind, future stock assessments should at look at different sex ratio scenarios to provide upper and lower bounds of exploitation and current stock status.

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## BIOGRAPHICAL SKETCH

Geoffrey H. Smith Jr. was born in Sarasota, Florida. He grew up in this same city, and spent much of his younger years at sea on his parent's commercial grouper fishing vessel. The time spent with his parents on their vessel is what started a lifelong love of the sea. His interest with the ocean and all of its organisms grew as he did. Geoffrey became an avid fisherman and aquarist, who collected most of his own specimens. He attended Sarasota High School and graduated 2002. While in high school he spent the majority of his summer vacation volunteering for the Stock Enhancement Program at Mote Marine Laboratory. He continued this work for two summers after his high school graduation as a sub-contractor.

In the fall of 2002 Geoffrey began his undergraduate studies at New College of Florida. While at New College he worked as a lab assistant at the Pritzker Marine Biology Research Center. He also acted as a teaching assistant for a number of classes and co-taught a field course on local marine fauna. Geoffrey's studies at New College culminated in a thesis focusing on spatial mapping in an intertidal fish species. In 2006 he earned his B.A. in Marine Biology. Upon graduating he continued to work at New College as a lab assistant at the Pritzker lab and for the chemistry department.

In 2008, Geoffrey began work as a lab technician in both the Philips lab and Murie/Parkyn lab in the Program for Fisheries and Aquatic Sciences at the University of Florida. His work in the Murie/Parkyn lab lead to a position as a M.S. graduate assistant in the fall of 2008. His studies concentrated on non-lethal sexing and sex ratio impacts on population dynamics in greater amberjack. Upon the completion of his masters research Geoffrey will begin a Ph.D. in Fisheries and Aquatic Sciences at the University of Florida, which will focus on early life history of common snook. Geoffrey plans to pursue a teaching and research position at a small to

moderate sized university where he will be able to work one on one with students while continuing to pursue his research interests.