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Abstract

Greater amberjack, *Seriola dumerili*, is managed in the United States as two separate stocks, the Gulf of Mexico and the US South Atlantic 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, since amberjack may be subject to sex-specific mortality resulting from current size regulations and possible skewing of the sex ratio towards one sex or the other in some regions. In addition to determining the sex, and hence sex ratio, of the landed catch, it would also be useful to non-lethally determine the sex of a fish prior to its release in a tagging study. This would facilitate the use of sex-specific data in assessing sex ratios, sex-specific migration patterns, and sex-specific mortality rates. To address this issue, a non-lethal method of sex determination was modified for use on greater amberjack. External features of the urogenital region were used to sex fish, and the use of urogenital catheterization was applied to verify sex and obtain oocyte samples from females. Of the 194 fish that had their sex verified, 193 were sexed correctly yielding an accuracy of 99.5%. Relative maturation status of females was determined from oocyte samples collected via urogenital catheterization. This allowed for the identification of individuals that were currently spawning or that would likely spawn in the upcoming spawning season. However, no differentiation could be made between immature and resting individuals. Analysis of published datasets, as well as the non-lethal sexing data from the current study, suggests that the Gulf stock likely has a male to female sex ratio in the range of 1:1 to 0.5:1, with estimates ranging from 0.4:1 to 1.1:1.

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). In the United States, this species is managed as two separate stocks, the US South Atlantic stock and the Gulf of Mexico stock (Gulf stock). Both of these stocks are subject to commercial and recreational fishing. Concerns about overfishing of the Gulf stock have resulted in increased regulation of both commercial and recreational fisheries since 1990 (Hood 2006), and the most recent assessment of this stock found it to be overfished and undergoing overfishing (NMFS 2006, 2011).

The 2006 stock assessment (NMFS 2006) and 2010 update (NMFS 2011) was based on the best available data, but there was still a substantial lack of adequate information available, which resulted in the use of some 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 it must be assumed that it is 1:1, as was the case in the 2006 stock

assessment (NMFS 2006). However, 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 (Harris et al. 2007; Murie and Parkyn 2008) and the minimum size limits placed on the fisheries (i.e., sex selectivity by the fishery). Disproportionate catches of one sex or 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 and bag limit regulations and voluntary releasing of fish (i.e., released or discarded fish are rarely sampled).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 releases and discards, rather than simply obtaining sex information by sampling a fraction of the landed catch.

The use of sex chromosomes from genetic samples can be used to non-lethally determine the sex in some species, but there are a number of species that lack sex chromosomes including

greater amberjack (Sola et al. 1997). A number of other non-lethal methods have also been developed to assess the sex and maturity in a number of species, including: analyzing steroid, hormone, and protein levels (Sangalang et al. 1978; Le Bail and Breton 1981; Gordon et al. 1984; Johnson and Casillas 1991; Heppell and Sullivan 1999; Webb et al. 2002; Evans et al. 2004; Feist et al. 2004); palpating the gonad through the stomach wall via insertion of a finger into the mouth (Kano 2005); surgical observation and biopsy of the gonads (Ritchie 1965; Alam and Nakamura 2008); endoscopy (both through the genital pore and incision of the abdominal wall) (Driscoll 1969; Moccia et al. 1984; Ortenburger et al. 1996; Kynard and Kieffer 2002; Wildhaber et al. 2005; Bryan et al. 2007; Swenson et al. 2007); ultrasonography (Martin et al. 1983; Reimers et al. 1987; Bonar et al. 1989; Mattson 1991; Shields et al. 1993; Blythe et al. 1994; Karlsen and Holm 1994; Martin-Robichaud and Rommens 2001; Moghim et al. 2002; Colombo et al. 2004; Evans et al. 2004; Wildhaber et al. 2005; Newman et al. 2008) urogenial catheterization (Shehadeh et al. 1972; Ross 1984; Garcia 1989; Alvarez-Lajonchère et al. 2001; Coward and Bromage 2001; Ferraz et al. 2004); and examining external urogenital features (Sigler 1948; McComish 1968; Parker 1971; Casselman 1974; Norton et al. 1976; Noltie 1985; Benz and Jacobs 1986; Murie 1991; St-Pierre 1992; Vecsei et al. 2003).

To apply a non-lethal sex determination method in the field, it would need to meet certain criteria, including: 1) be applicable throughout the year and over a range of sizes; 2) not require anesthesia since amberjack are harvested for consumption (Coyle et al. 2004; Kahn and Mohead 2010); 3) be relatively simple and quick to perform, as well as being minimally invasive allowing individuals to be released in good health; and 4) it would also be desirable if the method required minimal costs. Based on these criteria, non-lethal sexing through the use of external urogential features appeared to be a potentially viable method for greater amberjack.

The use of external urogenital features cannot, however, be used to obtain the maturational status of an individual. Urogenital catheterization also meets most of the criteria outlined above, but can potentially provide maturational status of an individual as well as validation of other sexing methods, such as the use of external urogenital features, through the collection of gonadal tissues or fluids.

The goal of this study was to determine if the combination of external urogential features and urogenital catheterization would allow for the non-lethal determination of sex and relative maturation status of female greater amberjack. Application of this method, as well as analysis of prior studies and published data, was used to develop a range of estimates for the overall sex ratio of the Gulf of Mexico stock of greater amberjack.

Methods

Sex Differentiation of Urogenital Pores

Initially, eight (6 males and 2 females) greater amberjack were collected as part of an ongoing tagging study (Murie et al. 2011) 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 three 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 urogential pore(s), and differences in the spacing, location, and general appearance of the urogenital pore(s) and surrounding tissues were noted.

Field-based Sex Identification by means of Urogenital Pores and Accuracy of Sex Determination

To apply the external sexing of amberjack to fish sampled in the field, and to determine the accuracy of the method, amberjack were sexed during tagging trips in 2009 (March, April, May and November) and 2010 (March, April, and June). Fish were caught with hook and line and bandit fishing gear off the coast of Florida (Little Torch Key, Madiera Beach, Suwannee, and Apalachicola) and Louisiana (Grande Isle). Fish were measured for fork length (FL, nearest mm), tagged below the anterior portion of the second dorsal fin with a Hallprint dart tag, and two pectoral fin rays 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, by means of the sexing differentiation criteria. The accuracy of sex determination with this method was based on validation 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.

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 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 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. 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). Milt samples were also obtained using this catheterization procedure in males that did not express milt on capture. All samples obtained from the catheter were placed in 20 ml scintillation vials containing 5 ml of a10% solution of phosphate buffered formalin (PBF). The catheter was flushed with deionized water until clean 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 only 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 by means of 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© Imaging System was used to measure the diameter of 50 oocytes or as many as possible when less than 50 measurable oocytes were 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 ooctytes, but no late developing oocytes) (Grau et al. 1996; Micale et al. 1999; Poortenaar et al. 2001; and Harris et al. 2004, 2007) (Table 1). The size frequencies of oocytes in these stages were plotted and compared with 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 from published literature or data sources, as well as by applying non-lethal sexing of fish collected in field-based

sampling as an alternative method. 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. In addition, sex ratios of fish based on 100-mm size classes were estimated. Size classes ranged from <700 mm FL for those fish close to or below the recreational size limit during the period of the tagging study (711 mm or 28 in FL) through size classes of fish vulnerable to recreational fishing (711 mm FL up to 2008 and 762 mm or 30 in FL thereafter) and commercial fishing (≥ 914 mm or 36 in FL). The sex ratios of fish ≥1000 mm FL were analyzed as a separate group because a number of previous studies have indicated 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 (Murie et al. 2011) were analyzed. Sex ratios for fish sampled in the Gulf of Mexico were calculated in the same manner as for the Murie and Parkyn (2008) dataset for consistency.

228 Results

229 *Sex Differentiation by means of Urogenital Pores*

230 Urogenital pores of both male and female greater amberjack were surrounded by white,
231 papilla-like folds of tissue (Figure 2). In addition, both males and females were found to have
232 separate urinary and genital pores. However, the positions of these pores in relation to one
233 another were different. In males, the genital pore lies along the midline with the urinary pore
234 located directly posterior to it. The two pores are separated from one another by a thin (generally
235 ≤1 mm), flesh-colored septum (Figure 2A). The septum dividing the two pores extended across
236 the urinary pore and on insertion of a probe into the urinary pore it generally covers the genital
237 pore and vice versa, making it difficult to observe both pores at one time. In females, both the
238 genital and urinary pores were observed to lie along the midline or to have one pore lie along the
239 midline and one pore to be positioned slightly off-center. The two pores were separated by a
240 greater distance than in males (generally > 1 mm), and in most cases the tissue between the pores
241 was at least partially white in color (Figure 2B). In some cases the white, papilla-like folds of
242 tissue that surround the urogenital pores extended between the two pores in females. The greater
243 separation of the pores in females allowed for easier viewing of both pores simultaneously, even
244 upon insertion of a probe, compared with males. Observation of live mature females in spawning
245 condition revealed that their genital pore was much larger than that of males and was often
246 crescent-shaped (Figure 2C).

247

248 *Field-based Sex Identification by means of Urogenital Pores and Accuracy of Sex Determination*

249 A total of 379 greater amberjack were sexed in the field via characters associated with the
250 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 (Figure 3).

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. The one individual that was incorrectly sexed in the field was a female that was sacrificed, and she was correctly sexed in the lab with urogenital pore characteristics prior to direct observation of her gonads via dissection. Both male and female greater amberjack of all sizes (ranging from 534 to 1412 mm FL) were accurately sexed, except for the one female that was 636 mm FL (Figure 4).

Maturation Staging of Females by means of 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 5A), in stages of early development (Figure 5B), in late stages of development or ripe and spawning (Figure 5C), and spent (Figure 5D). Of the 97 catheter samples obtained from females, 92 could be staged (Table 2) according to the criteria outlined in Table 1. Females catheterized ranged in size from 534 mm FL to 1412 mm FL (Table 3) and maturity stages of early development and late development could be differentiated in females as small as 800 mm FL (Table 3). In addition, a number females larger than 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 and 3).

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 6). 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 probably 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 4). 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 7). Beasley (1993) and Thompson et al. (1999, which include Beasley's data) have previously reported an overall moderately female-skewed sex ratio for greater amberjack off Louisiana (Table 4).

Estimates of sex ratios for fish less than 700 mm FL and for those greater than or equal to 700 mm FL from the Gulf of Mexico were relatively similar to their corresponding overall sex ratios (Table 4). Sex ratios based on the dataset of Murie and Parkyn (2008) indicated a female-

skewed sex ratio for all sizes of fish, while those from non-lethal sexing were slightly male-skewed.

Sex ratio estimates for fish greater than 1 m FL were female skewed for the non-lethal sexing of fish in the Gulf of Mexico, 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 larger than 1 m FL have also shown female skewing, both in the Gulf of Mexico (Figure 4) and the US South Atlantic (Harris et al. 2007). Overall, the average male to female sex ratio for fish greater than 1 m FL in the Gulf of Mexico was 0.43 ± 0.02 ($\pm$ SE).

Discussion

The use of urogenital pore characteristics to non-lethally sex greater amberjack in the field yielded high accuracies (99 and 100% for females and males, respectively) that are comparable to or greater than accuracies found in studies using this and other non-lethal sexing methods. The method of sex determination used for greater amberjack in this study was adapted from 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

318 method of sexing greater amberjack by means of urogenital characteristics was accurate
319 regardless of sex or size of fish. Perhaps, the greatest limitation of applying the method was that
320 fish less than 500 mm FL had such small urogenital pores that no attempt was made to sex them.
321 Although it was not observed in this study, small urogenital pores may also contribute to
322 incorrectly sexing fish between 500 and 700 mm FL, particularly during the initial training and
323 application of this method. The most common mistake would probably be to misidentify
324 immature females in this size range as being males due to the female's pores being smaller and
325 having less separation than seen in larger females or mature females within this size range. The
326 use of a magnifying glass may improve this method for these smaller individuals, but it may be
327 that the differences observed in larger fish have not fully developed in smaller individuals. The
328 use of dyes applied to the urogenital region can improve the sexing of some species, but for these
329 species the difference is in the number of pores in each sex and not the position of the pores
330 relative to one another (Rakocy and McGinty 1989; Popma and Masser 1999).

331 The general maturation stage of female greater amberjack was easily obtained by
332 examining oocyte samples extracted by means of urogenital catheterization. This was not an
333 unexpected outcome as urogenital catheterization has been used in monitoring egg maturation of
334 this species in prior studies on captive spawning (Kožul et al. 2001; Mylonas et al. 2004). The
335 upper end of the size frequencies of the oocytes measured in this study tended to be slightly
336 larger than those given in Grau et al. (1996), Micale et al. (1999), and Harris et al. (2007), which
337 may have resulted from regional differences in egg diameters or from differences in preparation
338 of the samples. Oocyte diameters in this study were obtained from whole preserved oocytes,
339 while those from the previous studies were obtained from histological sections that may have
340 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 indicates that the classification of an individual to a particular maturation stage based on classification of oocytes types by 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 with the use of 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 with 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 (Murie et al. 2011) 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. As with greater amberjack, tagging studies of other species would benefit from prior knowledge of sex. 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). 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).

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, however, showed minor male-skewing. These were similar to sex ratios obtained by Harris et al. (2007) in

387 the US South Atlantic, as well as to a value of 1:1, which is currently the assumed sex ratio for
388 assessments of the Gulf stock (NMFS 2006).

389 Sex ratios for greater amberjack greater than 1 m FL from both the Gulf of Mexico
390 (Beasley 1993; Thompson et al. 1999, current study) and the US South Atlantic (Harris et al.
391 2007; Smith 2011) indicated that there was a relatively large female-skew (approximately 70%
392 female) for this size class. This lends support to the notion that the commercial amberjack
393 fishery, which has a minimum size limit of 914 mm FL, probably has a higher selectivity for
394 female fish. The female-skewing observed in these larger individuals could arise from faster
395 growth rates that have been observed in female greater amberjack (Harris et al. 2007, Murie and
396 Parkyn 2008), or it could be attributable to some other factor such as greater natural mortality of
397 male greater amberjack.

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

404 There were potential biases and errors that may have occurred with both methods used to
405 calculate sex ratios in this study. The majority of the Murie and Parkyn (2008) dataset contained
406 samples obtained through port sampling. These port sampled fish may not accurately represent
407 the true sex ratio of the stock because port sampled fish do not represent the entire catch, but
408 only the portion of the catch that is brought to port. This provides no sex data for any of the
409 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. 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 (Smith 2011). 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 greater than or equal to 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. Differences in the sex ratios for fish less than 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 (Smith 2011). This may have lead 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 (Smith 2011), 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 in this study would at least represent a range of probable values that should be considered in the assessment of this stock.

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Table 1.—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

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Table 2.—Number of catheterized female greater amberjack classified into each maturation stage described in Table 1 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

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Table 3.—Number of catheterized female greater amberjack classified into each maturation stage described in Table 1 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

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Table 4—Overall sex ratios, sex ratios for individuals <700 mm fork length (FL), sex ratios for individuals ≥700 mm FL, and sex ratios for individuals ≥1000 mm FL for greater amberjack in the Gulf of Mexico.

Group	Sex ratio (m:f)	Sample size	Source
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)
≥700 mm FL	0.56:1	1233	Murie and Parkyn 2008 dataset (This study)
	1.19:1	210	Non-lethal sexing (This study)
≥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)

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764 Figure Legends

765

766 Figure 1.—Catheter used to obtain milt and oocyte samples from greater amberjack.

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768 Figure 2.—Urogenital region of greater amberjack with anus, genital pore, and urinary pore
769 denoted. The urinary pore is the most posterior structure. A) Male greater amberjack. B) Female
770 greater amberjack. C) Reproductively active female greater amberjack.

771

772 Figure 3.—Numbers of greater amberjack non-lethally sexed and numbers of greater amberjack
773 that had their sex verified by milt expression, urogenital catheterization, or disssection of
774 sacrificed fish.

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776 Figure 4.— Percent accuracy of non-lethal sexing of greater amberjack by means of urogenital
777 pore features by size class. Sample sizes are given above the respective bars for each size class.

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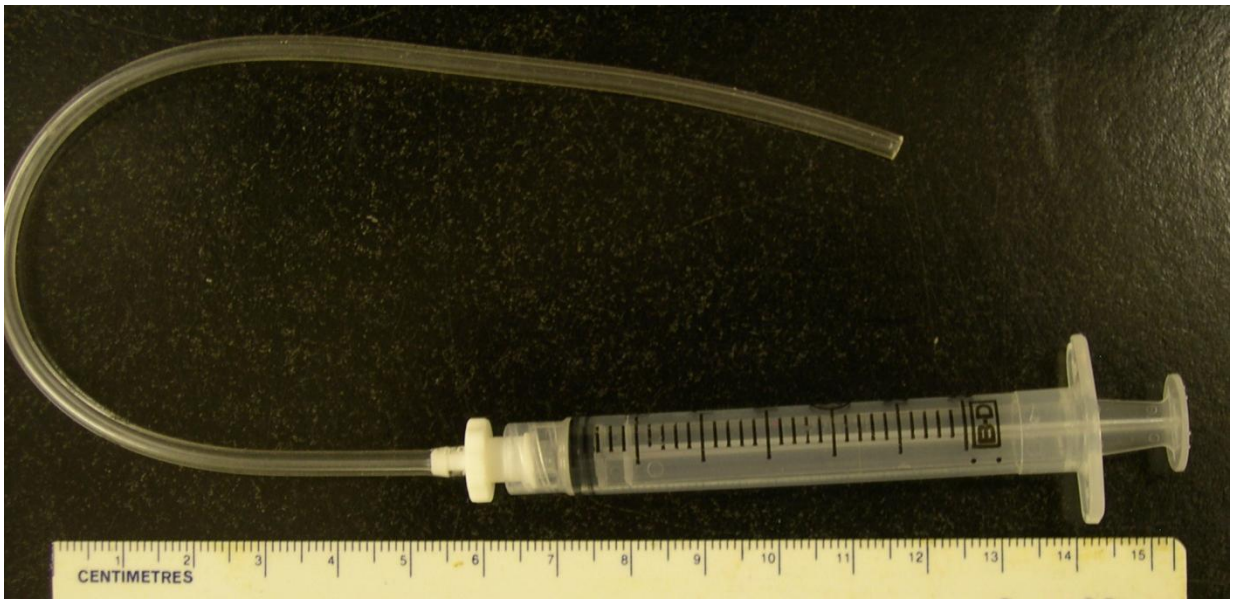
779 Figure 5.— Representative images of greater amberjack oocytes at various stages of maturity
780 collected via urogenitalcatheterization: A) female classified as immature or resting (only primary
781 oocytes visible = P); B) female classified as early developing (oocytes up to cortical alveolus
782 stage present = ED); C) female classified as ripe (contains fully hydrated oocytes = H, yolk
783 granule stages are also present = LD); D) female classified as spent (with degraded oocytes = D,
784 but no yolk granule or hydrated oocytes). Scale bar in all images is 0.5 mm.

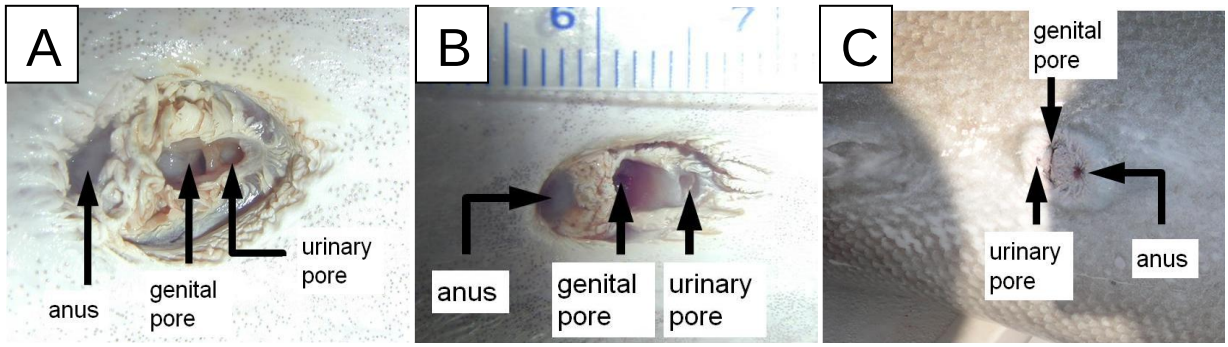
785

786 Figure 6.— Mean oocyte diamter of each type of measured oocyte from urogenital catheter
787 samples of greater amberjack following descriptions given in Table 1. Mean diameters were
788 calculated for each fish in which representative oocyte types were measured and then averaged
789 across all fish in which a particular type of oocyte was measured. Error bars represent the
790 standard error for the mean diameter of each oocyte type with the sample size being the number
791 of fish in which a particular type of oocyte was measured. Samples sizes are given above the
792 respective bars for each oocyte type.
793
794 Figure 7.— Annual male to female sex ratios from the Murie and Parkyn (2008) dataset for
795 2002-2008. The solid line represents the mean and the dashed line represents the median (2nd
796 quartile). Upper and lower ends of the box represent the 1st and 3rd quartiles, respectively.
797 Whiskers represent the upper and lower range of values observed on an annual basis.



Geoffrey Smith

Figure 1.



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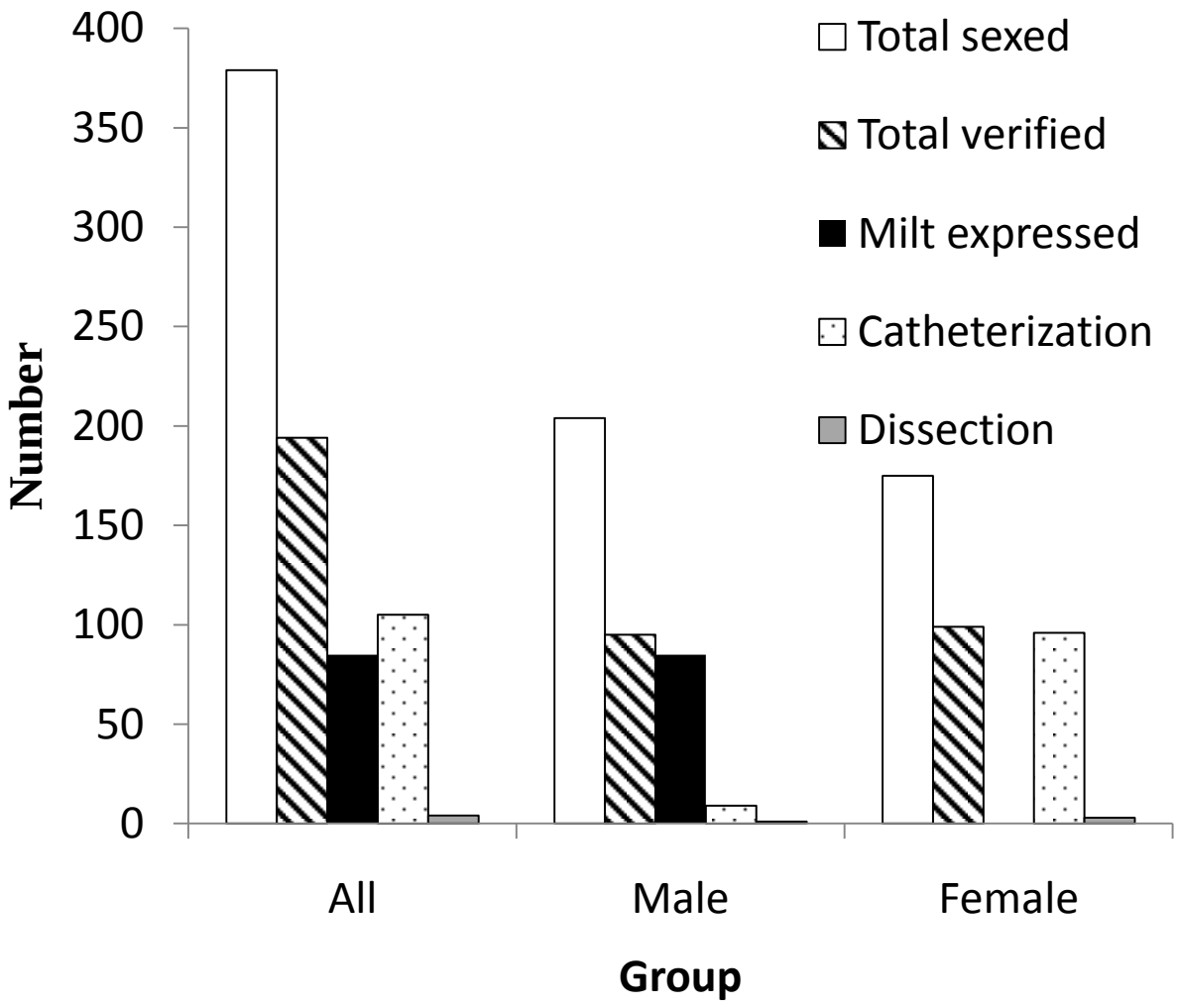

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Figure 3.

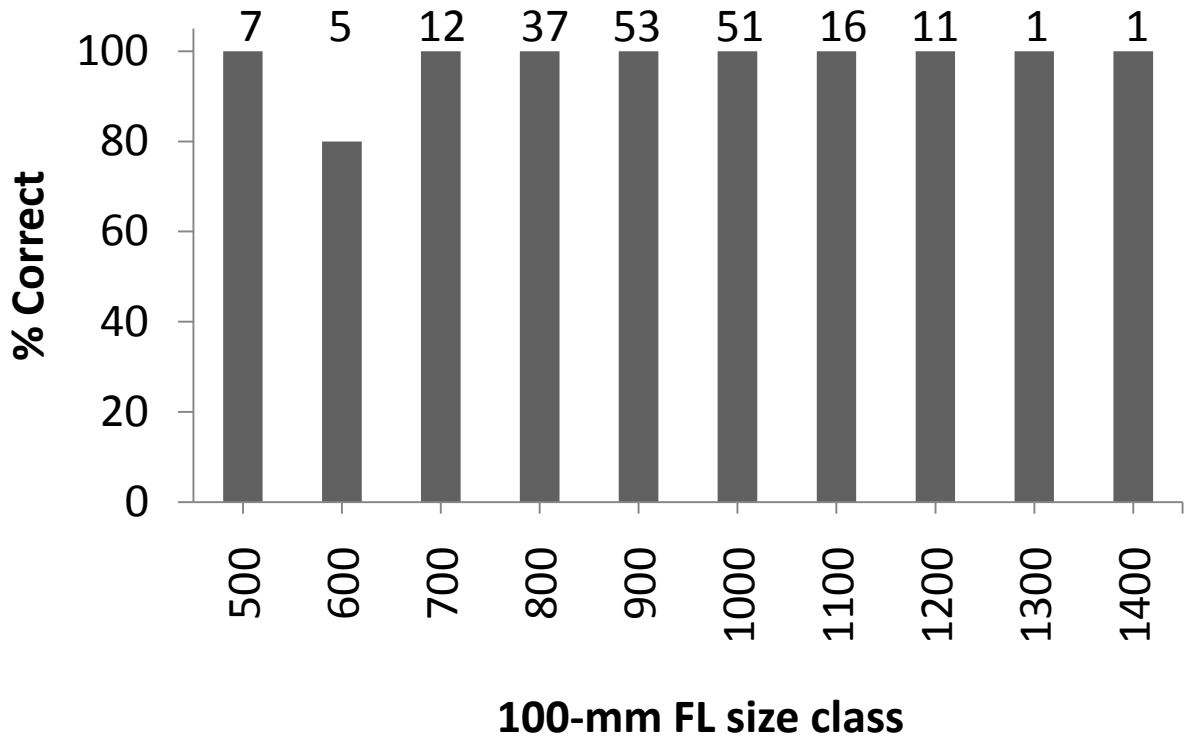

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Figure 4.

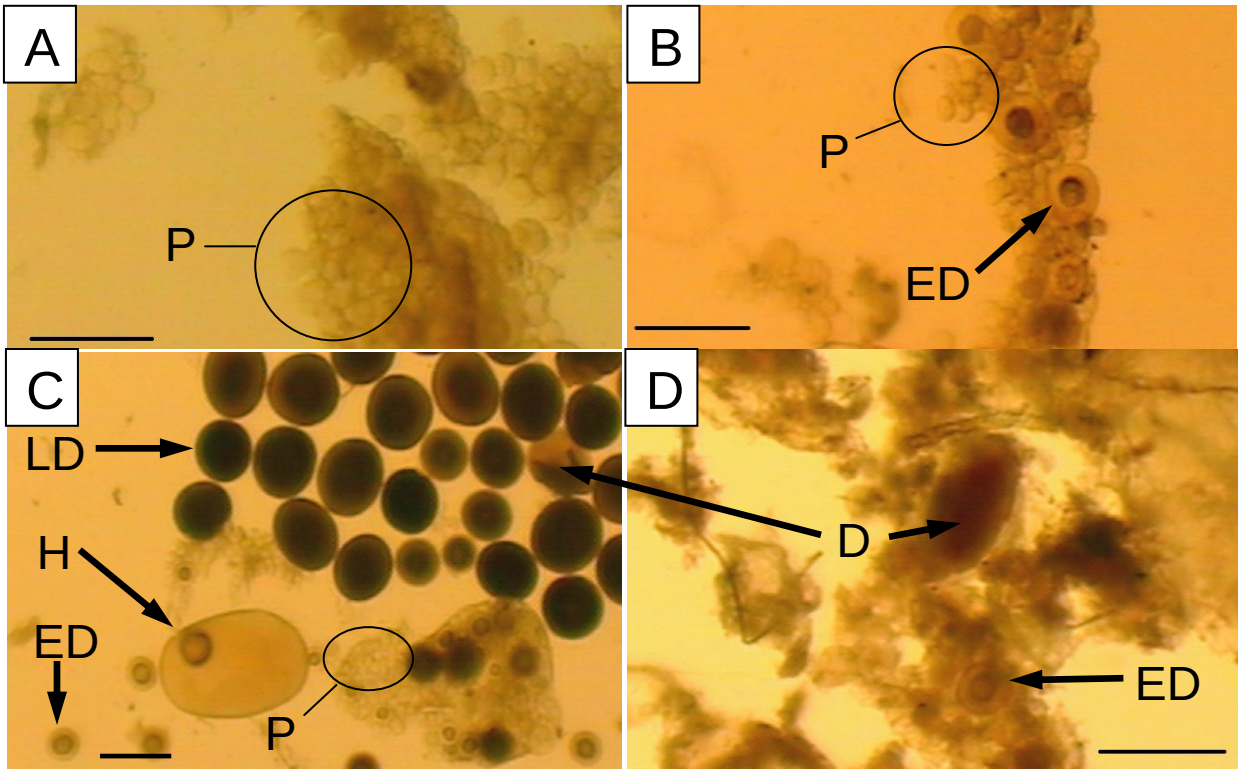

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Figure 5.

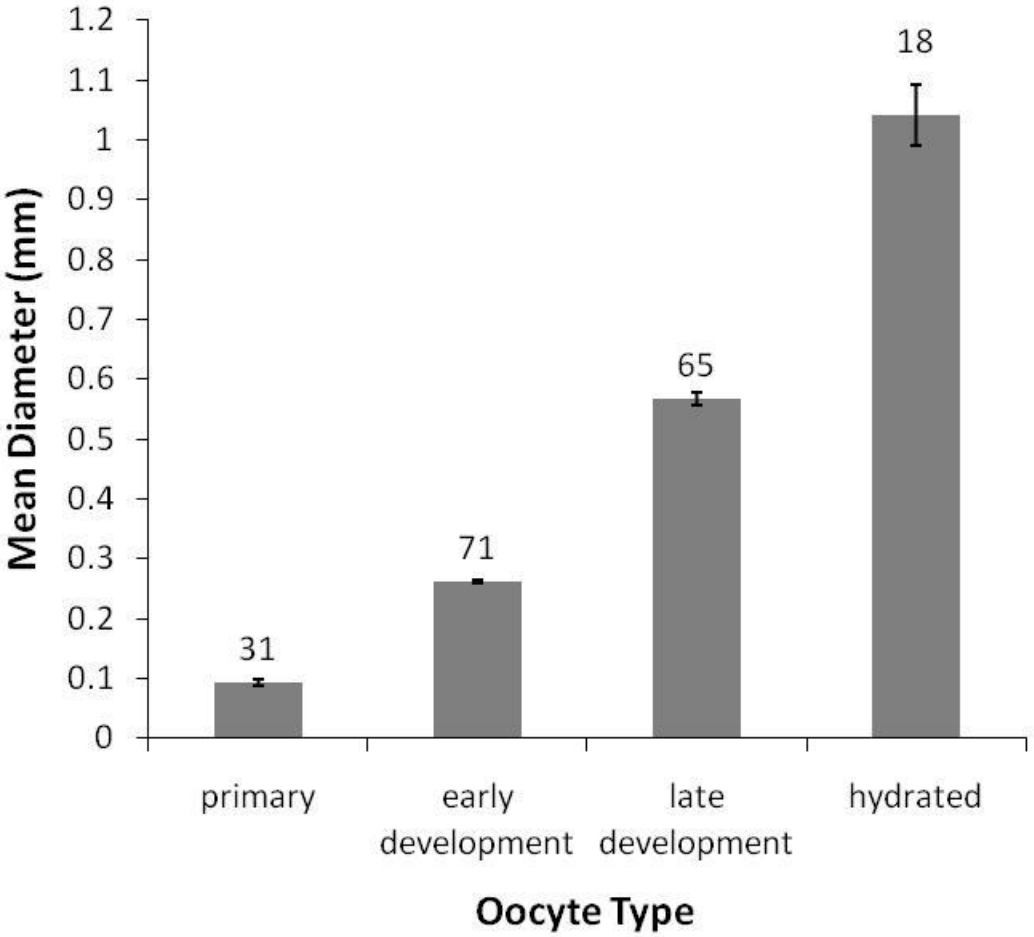

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Figure 6.

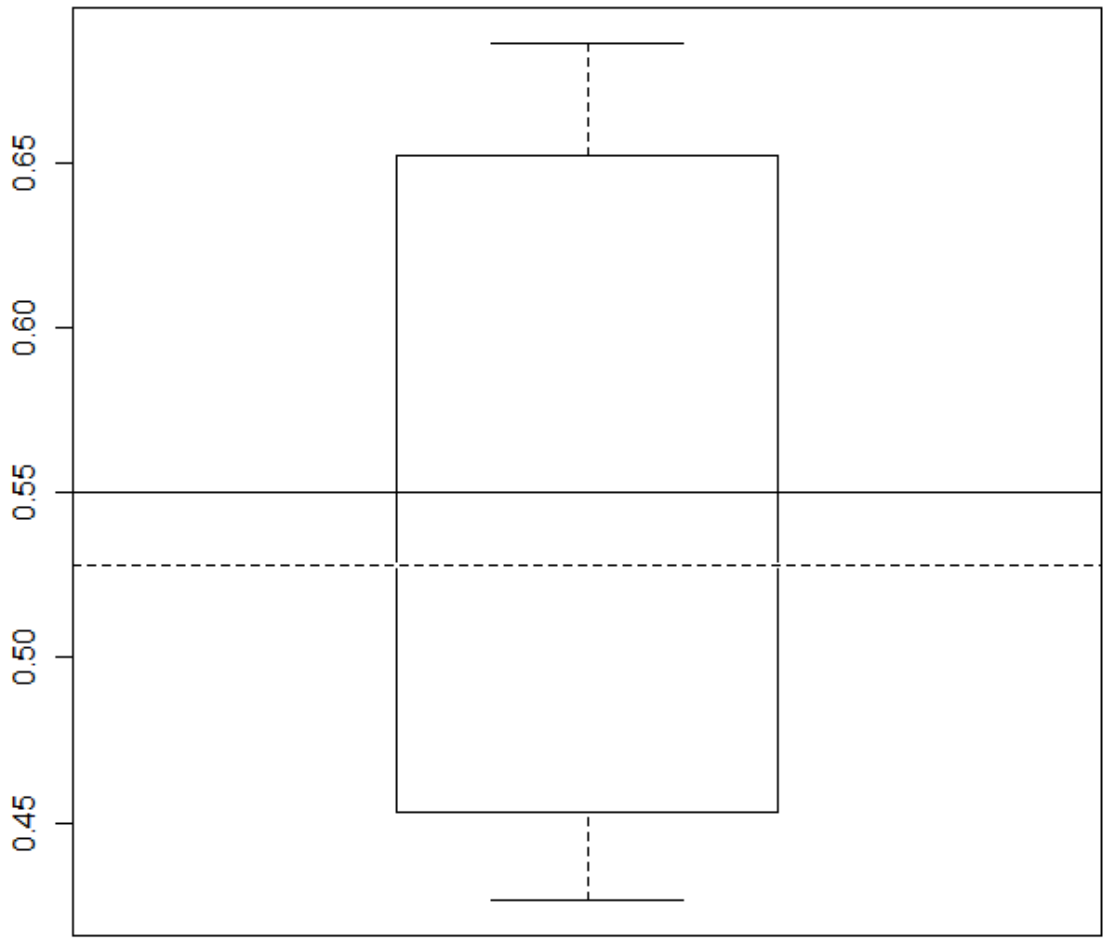

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Figure 7.